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Aryloxy Triester Phosphoramidates as Phosphoserine Prodrugs- A Proof of Concept Study

Ageo Miccoli,^[a] Binar A. Dhiani,^[a] Peter J. Thornton,^[b] Olivia A. Lambourne,^[a] Edward James,^[a]
Hachemi Kadri,^[c] and Youcef Mehellou*^[a]

- [a] Dr. Ageo Miccoli, Dr. Binar A. Dhiani, Ms. Olivia A. Lambourne, Dr. Edward James, and Dr. Youcef Mehellou
School of Pharmacy and Pharmaceutical Sciences
Cardiff University
King Edward VII Avenue, Cardiff University, CF10 3NB, U.K.
E-mail: MehellouY1@cardiff.ac.uk
- [b] Dr. Peter J. Thornton
Technology Solutions GBU
Solvay Solutions, Oldbury B69 4LN, U.K.
- [c] Dr. Hachemi Kadri
Department of Chemistry
Durham University
South Road, Durham, DH1 3LE, UK.

Supporting information for this article is given via a link at the end of the document.

Abstract: The specific targeting of protein-protein interactions by phosphoserine-containing small molecules has been scarce due to the dephosphorylation of phosphoserine and its charged nature at physiological pH, which hinders its uptake into cells. To address these issues, we herein report the synthesis of phosphoserine aryloxy triester phosphoramidates as phosphoserine prodrugs, which are enzymatically metabolized to release phosphoserine. This phosphoserine masking approach was applied to a phosphoserine-containing inhibitor of 14-3-3 dimerization, and the generated prodrugs exhibited improved pharmacological activity. Collectively, this provided a proof of concept of the masking of phosphoserine with biocleavable aryloxy triester phosphoramidate masking groups as a viable intracellular delivery system for phosphoserine-containing molecules. Ultimately, this will facilitate the discovery of phosphoserine-containing small molecule therapeutics.

Protein phosphorylation at serine residues is a fundamental phenomenon that is used by cells to affect the function, localization and degradation of proteins.^[1] In some cases, phosphorylated serine residues mediate protein-protein interactions via the docking of the phosphoserine residue into a positively charged pocket within the partner protein. An example of this is the serine phosphorylation of the adaptor protein 14-3-3, which facilitates its homodimerization.^[2] Attempts at inhibiting these phosphoserine-mediated protein-protein interactions with small molecules have mostly led, by design, to small molecules that lack phosphoserine. The move to discard phosphoserine groups from these molecules was driven by the fact that phosphoserine carries two negative charges at physiological pH, which limit its (passive) cellular uptake.^[3] Additionally, phosphoserine is also subject to dephosphorylation by alkaline phosphatases, a process that yields serine-containing derivatives that do not often retain potent pharmacological activity compared to their parent phosphoserine-containing molecules. Although the

masking of the phosphate group of phosphoserine was previously investigated, the study was limited to a phosphoserine mimetic (difluoromethylenephosphoserine) and not the natural phosphoserine moiety.^[4] With this in mind and in order to improve the drug-like properties of phosphoserine-containing small molecules, we explored the application of the aryloxy triester phosphoramidate technology to phosphoserine. This technology has been widely used to mask the 5'-O-monophosphate groups of nucleotides, and has so far led to two FDA-approved drugs; sofosbuvir and tenefovir alafenamide.^[5]

As a proof of concept, we initially synthesized phosphoserine with the aryloxy triester phosphoramidate masking groups (**Figure 1a**). For this, the *N*- and *C*-terminals of phosphoserine had to be protected^[6] first to allow for the selective addition of the aryloxy triester phosphoramidate moiety to the side chain hydroxyl group. The synthesis was initiated by the chlorination of *tert*-butanol (**1**) by copper(I) chloride with the peptide coupling reagent *N,N'*-dicyclohexylcarbodiimide (DCC).^[6] The generated compound, **2**, was then reacted with the commercially available *N*-Boc *L*-serine, **3**, in DCM to yield the *N*- and *C*-protected *L*-serine **4**.^[6] This latter compound was subsequently reacted with phenyl *L*-alanine methyl ester phosphorochloridate (**7**), which had been synthesized according to reported procedures^[7] by reacting *L*-alanine methyl ester hydrochloride (**6**) with the commercially available phenyl dichlorophosphate (**5**) in DCM and in the presence of triethylamine (NEt₃). This reaction yielded the desired phosphoserine aryloxy triester phosphoramidate, compound **8**, as a white solid in a good yield (62%).

With the phosphoserine aryloxy triester phosphoramidate in hand, we first studied whether the aryl and amino acid ester groups that mask the phosphate group of phosphoserine could be metabolized *in vitro* to release the unmasked phosphoserine species. It is now well established that the metabolism of the aryloxy triester phosphoramidate moieties is initiated by the cleavage of the ester motif by carboxypeptidase Y to yield

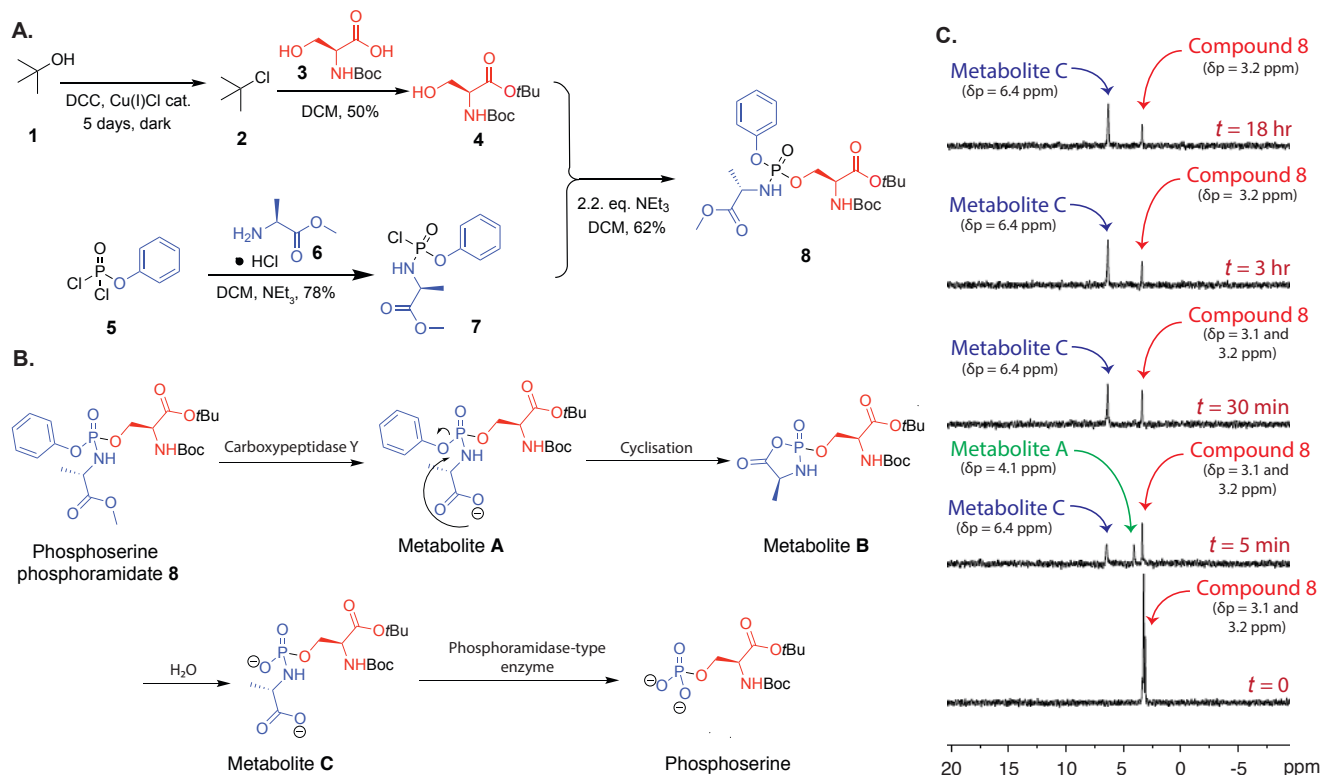


Fig. 1. Synthesis and *in vitro* esterase-mediated metabolism of phosphoserine aryloxy triester phosphoramidates. **A.** Synthesis of a phosphoserine aryloxy triester phosphoramidates (**8**). **B.** Mechanism of aryloxy triester phosphoramidates metabolism. **C.** ³¹P-NMR *in vitro* enzymatic assay of the breakdown of the phosphoserine phosphoramidate by carboxypeptidase Y.

metabolite **A** (Figure 1b).^[5a, 8] The formed carboxylate group then performs a nucleophilic attack onto the phosphate group leading to the release of the aryl group and the formation of a highly unstable five-membered anhydride ring (metabolite **B**, Figure 1b). This is subsequently opened up by a water molecule to generate the phosphoramidate metabolite **C** (Figure 1b). Finally, the phosphoramidase-type enzyme Hint-1^[9] cleaves the P-N bond of metabolite **C** to release the unmasked monophosphate group. With this in mind, we incubated compound **8** at 37 °C with commercially available recombinant carboxypeptidase Y and monitored the sample by ³¹P-NMR (Figure 1c).

As shown in Figure 1c, at *t* = 0 min, the ³¹P-NMR of compound **8** shows a sharp two singlets (δ_p = 3.1 and 3.2 ppm), which correspond to the two diastereoisomers (*R* and *S*) of the phosphoserine aryloxy triester phosphoramidate. This is typical of the aryloxy triester phosphoramidates as they have a chiral phosphorous and most current synthetic routes yield these compounds as a mixture of two diastereoisomers.^[5a, 7b, 10] After 5 minutes incubation with recombinant carboxypeptidase Y, two new peaks appeared; one appeared briefly at δ_p = 4.1 ppm while another peak at δ_p = 6.4 ppm also appeared and remained throughout the rest of the experiment. This is typical for aryloxy triester phosphoramidates as previous studies using carboxypeptidase Y showed a similar pattern with the peak at δ_p = 4.1 ppm corresponding to metabolite **A** whilst that at δ_p = 6.4 ppm corresponding to metabolite **C** (Figure 1b).^[10-11] Notably, after 30 minutes incubation, only the ³¹P-NMR peak at δ_p = 3.2 ppm remained from the two ³¹P-NMR peaks of the parent compound **8**. This indicated that one of the diastereoisomers of phosphoserine phosphoramidate, which has a ³¹P-NMR that corresponds to δ_p = 3.1 ppm, is a better

substrate for the enzyme and thus was processed quicker. From 30 minutes onward, only two ³¹P-NMR peaks persisted; δ_p = 3.2 and 6.4 ppm. After 18 h, the peak at δ_p = 6.4 ppm became the major peak with only one of the peaks from the original phosphoserine phosphoramidate two singlets remained. Mass spectrometry analysis of the products generated from this *in vitro* enzymatic assay after 18 h confirmed the breakdown of the phosphate masking groups and the release of metabolite **C** (Supporting Figure S1). As in this *in vitro* assay, there was no phosphoramidase-type enzyme, metabolite **C** was not further processed to release the fully unmasked phosphoserine species. To predict whether metabolite **C** would be a good substrate for Hint-1, we performed *in silico* docking of the metabolite into the crystal structure of the human Hint-1 co-crystallized with AMP (PDB 1KPF) as previously reported.^[10] The results showed that metabolite **C** sits in the Hint-1 active site and the phosphate group of phosphoserine forms key interactions with catalytic residues of Hint-1 (serine107, histidine112 and histidine114) suggesting that it could be a good substrate for this enzyme (Supporting Figure S2).^[9b] These docking results are in line with previous docking studies for the same enzyme that predicted and verified whether the P-N bond of the docked substrates could be cleaved off by Hint-1.^[9b] Together, these results indicate that phosphoserine aryloxy triester phosphoramidates are metabolized in a similar fashion to the nucleoside monophosphate aryloxy triester phosphoramidates.

In order to establish the applicability of the aryloxy triester phosphoramidate approach in improving the pharmacological activity of phosphoserine-containing compounds, we applied this approach to a 14-3-3

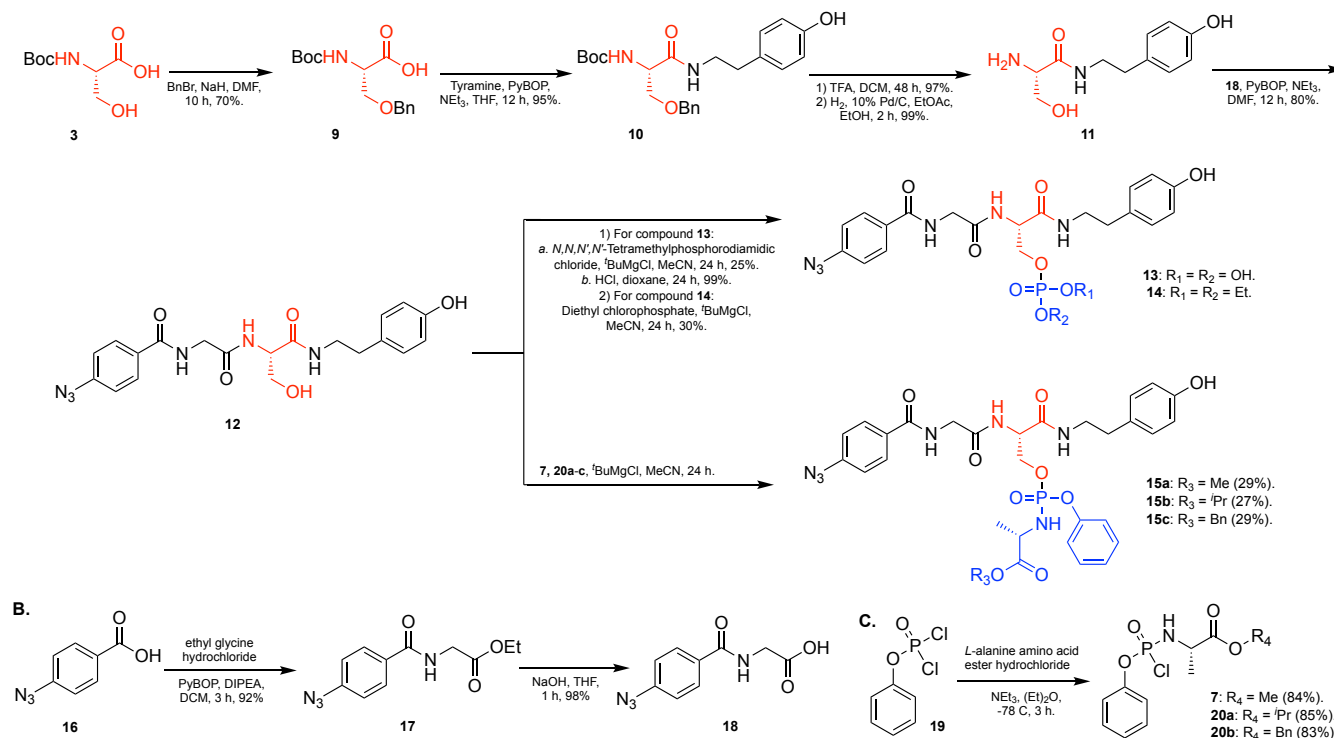


Fig. 2. A. Synthesis of the phosphoserine-containing 14-3-3 dimerization inhibitor (**13**), diethyl phosphate (**14**) and its aryloxy triester phosphoramidates (**15a-c**). The serine motif is shown in red while its phosphate and masked phosphate groups are shown in blue. **B.** Synthesis of the intermediate 2-((4-azidobenzoyl)oxy)acetic acid (**18**). **C.** Synthesis of phenyl L-alanine ester phosphorochloridates (**7** and **20a-b**).

dimerization inhibitor **13** (Figure 2), which showed promising, but not potent activity in cells.^[12] This compound is an ideal candidate for the application of the aryloxy triester phosphoramidate technology as it contains a phosphoserine motif that is essential for its pharmacological activity.^[12]

The synthesis of the aryloxy triester phosphoramidates of compound **13** commenced by the regioselective benzylation of the side chain hydroxyl group of *N*-Boc-*L*-serine (**3**), which proceeded with 70% yield (Figure 2a). The carboxylic acid of the product, **9**, was then conjugated to the amine group of tyramine to form a peptide bond using the peptide coupling reagent PyBOP in THF in the presence of NEt₃. This was then followed by sequential deprotection of the amine group and the side chain hydroxyl moiety using trifluoroacetic acid in DCM and hydrogenation, respectively. This resultant compound **11** was reacted with compound **18**, which had been generated^[13] in two steps from the commercially available 4-azidobenzoic acid (Figure 2b), to generate compound **12**.

For the synthesis of the reported 14-3-3 dimerisation inhibitor, **13**, compound **12** was reacted with *N,N,N',N'*-tetramethylphosphorodiamidic chloride in MeCN in the presence of the Grignard reagent *tert*butyl magnesium chloride (^tBuMgCl). The generated phosphorodiamidate derivative was then converted to the desired monophosphate species, **13**, by treatment with HCl in dioxane. We also synthesized the derivative of compound **13** where the phosphate group is fully masked by diethyl groups, compound **14**, to be used as a negative control in the biological assays. The synthesis was achieved by reacting compound **12** with diethyl chlorophosphate in the presence of ^tBuMgCl in MeCN to flourish the desired compound **14** in 30% yield. For the synthesis of the aryloxy triester

phosphoramidates of the 14-3-3 dimerization inhibitor, **13**, the synthesis was accomplished by coupling compound **12** to the appropriate phosphorochloridate (**7**, **20a** or **20b**, Figure 2c) in the presence of ^tBuMgCl in MeCN to give the desired aryloxy triester phosphoramidates (**15a-c**), as a mixture of two diastereoisomers, in low yields (~ 30%). Notably, the phosphorochloridates **7**, **20a-b** were synthesised as shown in Figure 2c and reported previously.^[7]

In order to determine the efficacy of the synthesized aryloxy triester phosphoramidate prodrugs to inhibit 14-3-3 dimerization and cell proliferation, we treated the lung cancer cell line, A549, with the 14-3-3 dimerization inhibitor, **13**, its diethyl phosphate derivative, **14**, and the aryloxy triester phosphoramidates **15a-c** as well as compound **12** at the indicated concentrations (12.5, 25, 50 and 100 μM) for 48 and 72 h (Figure 3). Cell viability was then determined using standard MTT assays. The results showed that, as expected, the unphosphorylated compound **12**, its phosphorylated derivative **13** and the diethyl phosphate compound **14**, did not show any significant effect on cell viability. Encouragingly, the aryloxy triester phosphoramidates **15a-c**, apart from **15a**, did show significant dose-dependent reduction of A549 cell viability (Figure 3). This is because these aryloxy triester phosphoramidates are neutral at physiological pH, unlike the parent compound **13**, and thus they are more readily taken up by cells via passive diffusion. The fact that among this series of aryloxy triester phosphoramidates, the isopropyl and benzyl ester phosphoramidates (**15b** and **15c**, respectively) showed significant biological activity and the methyl ester phosphoramidate, **15a**, did not demonstrate profound biological activity, is in line with the established structure-

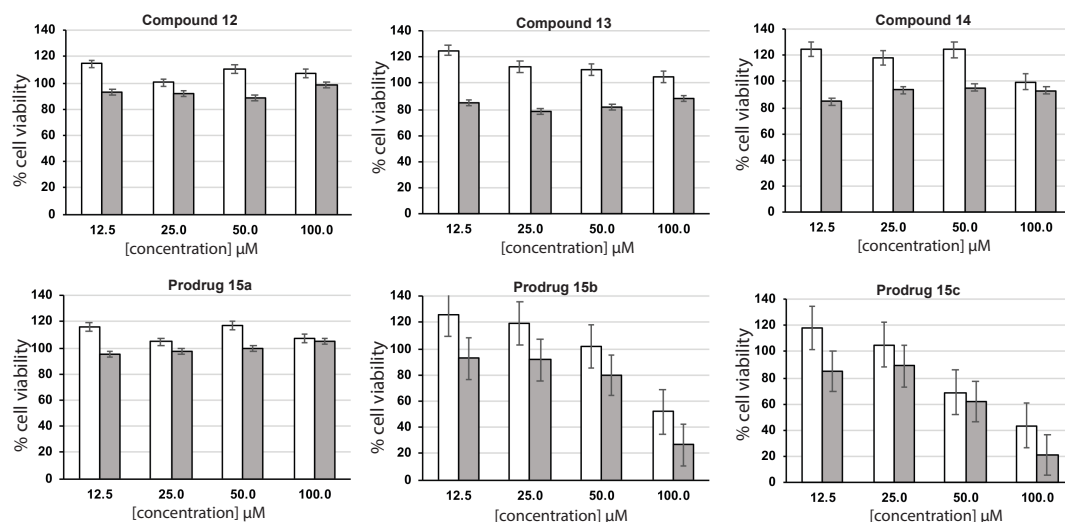


Fig. 3. Cell viability of the unmasked phosphoserine compound (**12**), phosphoserine-containing 14-3-3 dimerization inhibitor (**13**), its diethyl phosphate derivatives (**14**) and the aryloxy triester phosphoramidates (**15a-c**). Cell viability was determined by standard MTT assay. The compounds were incubated with A549 lung cancer cell line for 48 h (white bar) and 72 h (grey bar) at the indicated concentrations. The percentage of cell viability was calculated and presented as normalized value to control DMSO. Error bars show standard error from triplicate experiments.

activity relationship of aryloxytriester phosphoramidates of 5'-O-nucleoside monophosphates. [5a, 7b]

In summary, masking the phosphate group of phosphoserine with an aryl motif and an amino acid ester serves as a useful approach for the intracellular delivery of phosphoserine. Indeed, this work showed using a combination of *in vitro* enzymatic and *in silico* docking studies that these phosphoserine aryloxy triester phosphoramidates are metabolized to release the unmasked phosphoserine moiety. The application of this approach to a 14-3-3 phosphoserine-containing molecule significantly improved its pharmacological efficacy in cancer cells. Together, this validates this approach of phosphoserine masking with biocleavable motifs, and this will in the future facilitate the discovery of phosphoserine-containing molecules as powerful tool compounds and potential therapeutics.

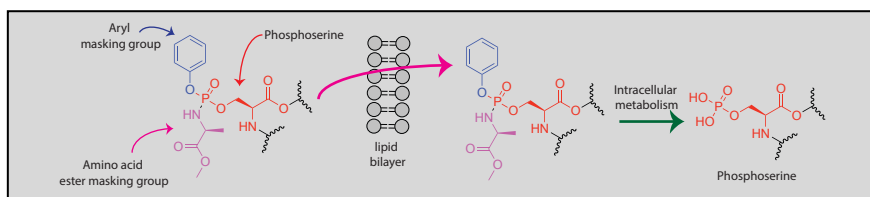
Experimental Section

Experimental details are found in the Supporting Information.

Keywords: Phosphoserine • Phosphoramidate • Prodrug • 14-3-3 • Cancer

- [1] a) T. Pawson, J. D. Scott, *Trends Biochem. Sci.* **2005**, 30(6), 286-290; b) T. Hunter, *Cell* **1995**, 80(2), 225-236; c) P. Cohen, *Trends Biochem. Sci.* **1992**, 17(10), 408-413.
- [2] M. B. Yaffe, K. Rittinger, S. Volinia, P. R. Caron, A. Aitken, H. Leffers, S. J. Gamblin, S. J. Smerdon, L. C. Cantley, *Cell* **1997**, 91(7), 961-971.
- [3] L. Xie, S. Y. Lee, J. N. Andersen, S. Waters, K. Shen, X. L. Guo, N. P. Moller, J. M. Olefsky, D. S. Lawrence, Z. Y. Zhang, *Biochemistry* **2003**, 42(44), 12792-12804.
- [4] A. Arrendale, K. Kim, J. Y. Choi, W. Li, R. L. Geahlen, R. F. Borch, *Chem. Biol.* **2012**, 19(6), 764-771.
- [5] a) Y. Mehellou, H. S. Rattan, J. Balzarini, *J. Med. Chem.* **2018**, 61(6), 2211-2226; b) Y. Mehellou, *ChemMedChem* **2016**, 11(11), 1114-1116.
- [6] S. Koch, D. Schollmeyer, H. Lowe, H. Kunz, *Chemistry* **2013**, 19(22), 7020-7041.
- [7] a) M. S. Davey, R. Malde, R. C. Mykura, A. T. Baker, T. E. Taher, C. S. Le Duff, B. E. Willcox, Y. Mehellou, *J. Med. Chem.* **2017**; b) L. Osgerby, Y. C. Lai, P. J. Thornton, J. Amalfitano, C. S. Le Duff, I. Jabeen, H. Kadri, A. Miccoli, J. H. R. Tucker, M. M. K. Muqit, Y. Mehellou, *J. Med. Chem.* **2017**, 60(8), 3518-3524.
- [8] a) G. Birkus, R. Wang, X. Liu, N. Kutty, H. MacArthur, T. Cihlar, C. Gibbs, S. Swaminathan, W. Lee, M. McDermott, *Antimicrob. Agents Chemother.* **2007**, 51(2), 543-550; b) J. Balzarini, A. Karlsson, S. Aquaro, C. F. Perno, D. Cahard, L. Naesens, E. De Clercq, C. McGuigan, *Proc. Nat. Acad. Sci. USA* **1996**, 93(14), 7295-7299.
- [9] a) J. Cheng, X. Zhou, T. F. Chou, B. Ghosh, B. Liu, C. R. Wagner, *Bioorg. Med. Chem. Lett.* **2009**, 19(22), 6379-6381; b) E. Murakami, T. Tolstykh, H. Bao, C. Niu, H. M. Steuer, D. Bao, W. Chang, C. Espiritu, S. Bansal, A. M. Lam, M. J. Otto, M. J. Sofia, P. A. Furman, *J. Biol. Chem.* **2010**, 285(45), 34337-34347.
- [10] Y. Mehellou, R. Valente, H. Mottram, E. Walsby, K. I. Mills, J. Balzarini, C. McGuigan, *Bioorg. Med. Chem.* **2010**, 18(7), 2439-2446.
- [11] M. Slusarczyk, M. H. Lopez, J. Balzarini, M. Mason, W. G. Jiang, S. Blagden, E. Thompson, E. Ghazaly, C. McGuigan, *J. Med. Chem.* **2014**, 57(4), 1531-1542.
- [12] H. Wu, J. Ge, S. Q. Yao, *Angew. Chem.* **2010**, 49(37), 6528-6532.
- [13] H. Garrido-Hernandez, K. D. Moon, R. L. Geahlen, R. F. Borch, *J. Med. Chem.* **2006**, 49(11), 3368-3376.

Entry for the Table of Contents



The development of phosphoserine-containing therapeutics has been limited by their poor drug-like properties. To address this, we applied the aryloxy triester phosphoramidate prodrug technology to phosphoserine and a phosphoserine-containing 14-3-3-dimerization inhibitor. These prodrugs exhibited improved biological activity highlighting the promise of this prodrug technology in the discovery of phosphoserine-containing therapeutic.

Institute and/or researcher Twitter usernames: [@Youcef_Mehellou](#), [@PharmacyCU](#)

SUPPORTING INFORMATION

Aryloxy Triester Phosphoramidates as Phosphoserine Prodrugs- A Proof of Concept Study

Ageo Miccoli,^[a] Binar A. Dhiani,^[a] Peter J. Thornton,^[b] Olivia A. Lambourne,^[a] Edward James,^[a]
Hachemi Kadri,^[c] and Youcef Mehellou*^[a]

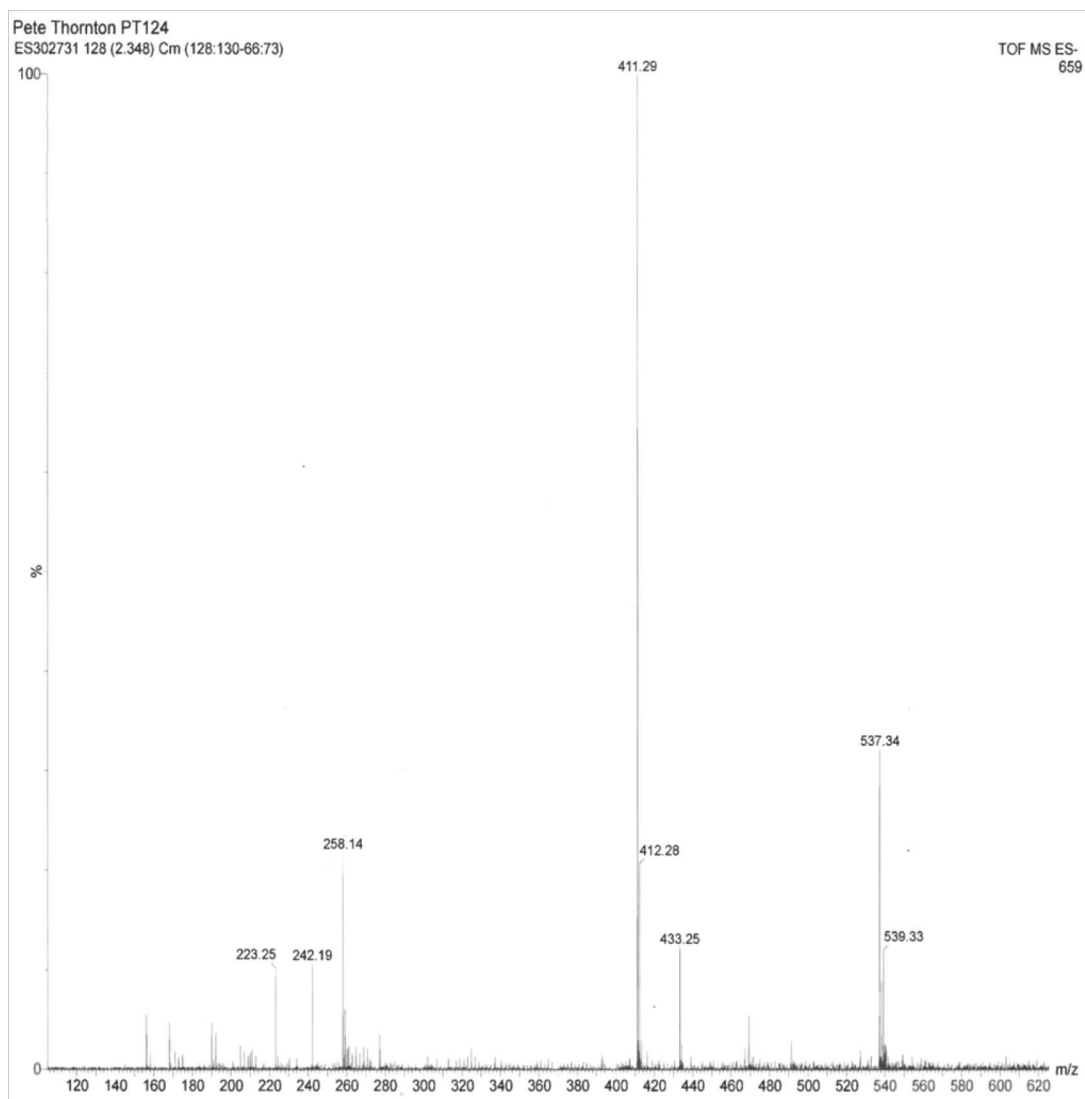
^a*School of Pharmacy and Pharmaceutical Sciences, King Edward VII Avenue, Cardiff University, CF10 3NB, U.K.*

^b*Technology Solutions GBU, Solvay Solutions, Oldbury B69 4LN, U.K.*

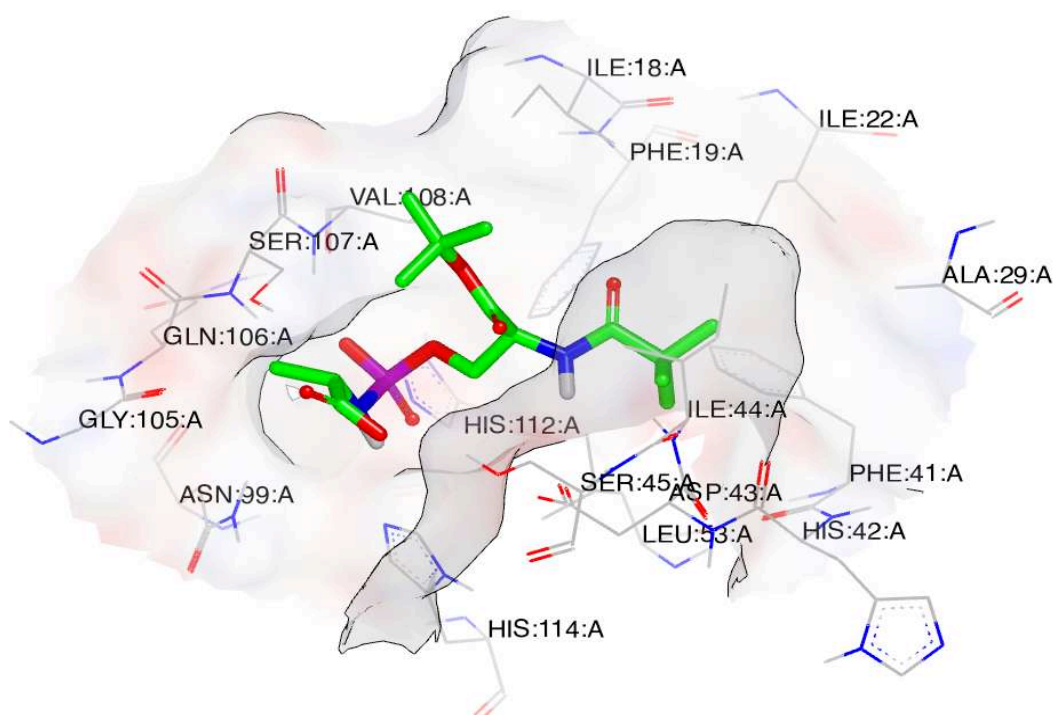
^c*Department of Chemistry, Durham University, South Road, Durham, DH1 3LE, UK.*

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I. SUPPORTING FIGURES



Supporting Figure S1. A negative mode mass spectrometry spectra of the mixture from compound **8** with carboxypeptidase Y after 18 h at 37 °C. The results show the presence of metabolite **C**, which has a mass of 412.3 g/mol.



Supporting Figure S3. Docking of metabolite **C** into the crystal structure of the human HINT1 (PDB 1KPF). The docking shows the close proximity of the phosphate group of metabolite **C** and its masking group to the HINT1 catalytic residues Ser107, His112 and His114.

II. REAGENTS AND METHODS

All solvents used were received from commercial suppliers. All of the other reagents used in the synthesis were purchased from Sigma-Aldrich, Fisher Scientific, Fluorochem, Alfa-Aesar or Carbosynth. All reactions were carried out under an argon or nitrogen atmosphere unless stated otherwise. All glassware was oven-dried overnight and allowed to cool under dry argon/nitrogen. Thin Layer Chromatography (TLC). Reactions were monitored with analytical TLC on Merck silica gel 60-F₂₅₄ precoated aluminium plates of 0.2 mm thickness. Plate visualisation was carried out with UV (254 nm), potassium permanganate stain (with heating) or phosphomolybdic acid stain (with heating). *Flash Column Chromatography*. Column chromatography was performed on silica gel (35–70 μ M) as a stationary phase and with the described solvent system. Glass columns were slurry-packed under gravity and fractions withholding product were identified by TLC analysis or ³¹P NMR analysis. *Nuclear Magnetic Resonance Spectroscopy*. NMR spectra data were recorded on a Bruker AVIII300 (¹H, 300 MHz; T = 295 K. ¹⁹F, 300 MHz; T = 295 K. ³¹P, 121 MHz; T = 295 K), AVIII400 (¹³C, 101 MHz; T = 295 K) or a Bruker Avance 500 (¹H, 500 MHz; T = 295 K. ¹³C, 125 MHz; T = 295 K. ¹⁹F, 407 MHz; T = 295 K. ³¹P, 202 MHz; T = 295 K) spectrometer in the deuterated solvents indicated where the spectra were calibrated on residual solvent peaks. Chemical shifts (δ) are quoted in ppm (parts per million), and *J* values are quoted in Hz. In reporting spectral data, the following abbreviations were used: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet) and m (multiplet). 1D ¹³C NMR spectra was recorded using PENDANT pulse sequences. *Mass Spectrometry*. EI mass spectra was recorded on a VG ZabSpec magnetic sector mass spectrometer by the analytical staff and facilities at the School of Chemistry, University of Birmingham. ES mass spectra was manually recorded on a Bruker microtof mass spectrometer, operating in positive or negative ion modes. High resolution mass spectrometry was performed as a service by

Bath University, using a 6545 LC/Q-TOF mass spectrometer. High Performance Liquid Chromatography. HPLC was carried out on a DIONEX summit P580 quaternary low-pressure gradient pump with a built-in vacuum degasser using a Summit UVD 170s UV/vis multichannel detector. Solvents were used as HPLC grade and not purified further. Chromeleon software was used to visualize and process the obtained chromatograms. Analytical separations used a flow rate of 1 mL/min. Unless otherwise stated, all tested compounds had a purity of $\geq 95\%$ as shown by HPLC.

Cell treatment and MTT assay. A549 cells were cultured in Ham's F12K (Kaighn's) medium (TermoFisher) supplemented with 10% Fetal Bovine Serum (FBS) (Gibco) and 1% of PenStrep (Gibco). The cells were maintained at 37 °C in the incubator with 5% CO₂ to reach 70-80% confluency to be used in the experiment. In 96-well plate, 50 μ L of complete growth media was added to each well. Each compound was prepared in a concentration of 400 μ M to reach the final concentration of 100 μ M for the highest concentration after several dilutions. Afterwards, 50 μ L of each compound solution was mixed with the 50 μ L of growth media in the first well. Serial dilution (2x dilution each step) was then performed by pipetting out 50 μ L of each compound solution from the first well and pipetted in the next well, which contains 50 μ L growth media and so on until the last well (11th well). The series of final concentration was ranging from 100 μ M to 0.10 μ M, and all compounds were prepared in triplicates. A 50 μ L of A549 cells containing 5,000 cells were added to each well, which contained 50 μ L of compounds solution in the growth medium. The 96 well plates were incubated at 37 °C in the incubator with 5% CO₂ for 48 and 72 hr before the MTT assay was performed. The MTT assay was performed using a CellTiter 96 Non-radioactive Proliferation Assay Kit (Promega). A 15 μ L dye solution was then added on each well. After incubation for 4 hr at 37 °C in the incubator with 5% CO₂, 100 μ L of stop solution was added to each well. The plates were then left overnight in room temperature before the absorbance was read on the microplate reader at 570 nm wavelength. As a control, DMSO was included in the experiments with 100% DMSO as the highest final concentration following serial dilution steps as the compounds. The same set of plates without cells (as a blank) were also measured its absorbance to be used for correcting the absorbance of the sample. Finally, the measured absorbance was then used for calculating the % cell availability by the formula:

$$\% \text{ cell viability} = \frac{\text{Absorbance sample} - \text{Absorbance blank}}{\text{Absorbance control DMSO}} \times 100\%$$

Carboxypeptidase Y Assay. The procedure was adapted from Mehellou *et al.*¹ Compound **8** (5.0 mg) was dissolved in acetone-d₆ (0.52 mL), followed by addition of Trizma buffer (0.64 mL, pH 7.4). After recording a control ³¹P NMR spectrum containing compound **8** in acetone-d₆ and buffer, defrosted Carboxypeptidase Y (0.3 mg dissolved in 0.27 mL of Trizma buffer), purchased from Sigma-Aldrich UK, was added to the mixture. A ³¹P-NMR was then run immediately after the addition and then at even time intervals over 11 hr. The sample was then analysed by ³¹P-NMR after 18 h. All ³¹P NMR spectra were recorded at 37 °C (± 1).

Molecular docking. PC Windows 7 with Intel Core i7-4790/3.6GHz microprocessor, 16 GB RAM, and 64 bit operating system was used to execute the computational studies. Docking was performed applying the molecular modelling modules, namely, Omega2, FRED, and VIDA provided by OpenEye Scientific Software (<http://www.eyesopen.com>). The three-dimensional crystal structure of the co-crystallized AMP with the human Hint1 was retrieved from the Protein Data Bank (PDB code 1KPF),² and the active site was subsequently identified on the basis of the bound ligand. Multiple conformers for metabolite C were generated by Omega2 using the default settings.³ FRED (fast rigid exhaustive docking) implements a rigid docking approach to fit these conformers into the predefined binding site and rank the poses by scoring functions.^{4, 5} The VIDA module was then used to

visualize and inspect the docked poses within the receptor's active site and to identify the main interacting residues.

III. SYNTHETIC PROCEDURES

***tert*-Butyl (tert-butoxycarbonyl)-*L*-serinate (4).** *tert*-Butanol (3.13 g, 42.23 mmol), DCC (6.10 g, 29.56 mmol) and copper(I)-chloride (0.031 g, 0.000313 mmol) were stirred under exclusion of light at 30 °C for 5 days to generate 2-chloro-2-methylpropane (2). The mixture was then diluted with anhydrous dichloromethane (20 mL) and a solution of *N*-*tert*-butyloxycarbonyl-*L*-serine (3) (2.0 g, 9.746 mmol) in anhydrous dichloromethane (16 mL) was added at 0 °C. The mixture was then allowed to warm to ambient temperature and stirred overnight. The solvent was subsequently removed *in vacuo* and the crude underwent flash chromatography (ethyl acetate:hexane 0:1 to 3:2) to yield compound 4 as a crystalline solid (1.272 g, 50%). δ_{H} (500 MHz, CDCl_3) 5.44 (s, 1H), 4.28 (s, 1H), 3.92 (m, 2H), 2.41 (m, 1H), 1.51 (s, 9H), 1.47 (s, 9H). δ_{C} (500 MHz, CDCl_3) 176.56, 169.70, 82.74, 80.36, 64.19, 56.44, 28.31, 28.01. MS (ES+) m/z 262.20 $[\text{M}+\text{H}]^+$ and 284.18 $[\text{M}+\text{Na}]^+$.

Methyl (chloro(phenoxy)phosphoryl)-*L*-alaninate (7). To a stirring solution of the commercially available phenyl dichlorophosphate 5 (0.35 mL, 2.370 mmol) and anhydrous DCM (10 mL), in a flame dried flask, was added *L*-alanine methyl ester hydrochloride 6 (301 mg, 2.155 mmol) and stirred for 15 minutes at room temperature, under an argon atmosphere. Anhydrous triethylamine (0.62 mL, 4.262 mmol) was added dropwise to the yellow solution at -78 °C to form a suspended white precipitate. The reaction mixture was stirred for 30 minutes before being left to stir and warm to room temperature for 2.5 hours. Upon reaction completion, the colourless solution was concentrated under reduced pressure and filtered with anhydrous diethyl ether. The yellow filtrate was concentrated under reduced pressure to afford a yellow oil, which was purified with column chromatography (ethyl acetate:hexane 6:4) to yield compound 7 as a colourless oil (505 mg, 84%). δ_{H} (300MHz, CDCl_3) 7.37 (dd, $J = 7.2, 2.1$ Hz, 2H, *H*-3), 7.29 – 7.23 (m, 3H, *H*-2 + *H*-4), 4.24 – 3.93 (m, 2H, *H*-5 + *H*-6), 3.78 (d, $J = 5.2$ Hz, 3H, *H*-9), 0.97 (dd, $J = 6.4, 3.0$ Hz, 3H, *H*-7). δ_{P} (121MHz, CDCl_3) 7.91, 7.55. Analytical data is in agreement with literature values.⁶

***tert*-Butyl-*N*-(tert-butoxycarbonyl)-*O*-(((*S*)-1-methoxy-1-oxopropan-2-yl) amino) (phenoxy) phosphoryl)-*L*-serinate (8).** To *tert*-butyl (tert-butoxycarbonyl)-*L*-serinate 4 (1.181 g, 4.519 mmol), methyl (chloro(phenoxy)phosphoryl)-*L*-alaninate 7 (1.255 g, 4.519 mmol) in anhydrous dichloromethane (40 mL) under nitrogen atmosphere was added trimethylamine (1.39 mL, 1.006 g, 9.942 mmol) dropwise and left to stir at ambient temperature overnight. The solvent was subsequently removed *in vacuo* and the crude underwent flash chromatography (ethyl acetate:hexane 0:1 to 3:2) to yield compound 8 as a white solid (1.398 g, 62%). δ_{P} (500 MHz, CDCl_3) 2.24, 2.12. δ_{H} (500 MHz, CDCl_3) 7.23 (m, 2H), 7.11 (m, 3H), 5.48 (m, 0.5H), 5.36 (m, 0.5H), 4.34 (m, 2H), 4.28 (m, 1H), 3.39 (m, 1H), 3.32 (m, 0.6H), 3.74 (m, 0.4H), 3.66 (s, 1.8H), 3.61 (s, 1.2H), 1.39 (s, 4H), 1.38 (s, 5H), 1.36 (s, 4H), 1.35 (s, 5H), 1.30 (m, 3H). δ_{C} (500 MHz, CDCl_3) 173.50, 168.25, 155.33, 150.65, 129.61, 124.88, 120.17, 82.69, 79.94, 67.40, 54.31, 52.49, 50.09, 28.28, 27.84, 20.86. MS (ES+) m/z 503.22 $[\text{M}+\text{H}]^+$ and 525.21 $[\text{M}+\text{Na}]^+$.

***O*-Benzyl-*N*-(tert-butoxycarbonyl)-*L*-serine (9).** Under a nitrogen atmosphere, *N*-(tert-Butoxycarbonyl)-*L*-serine 3 (500 mg, 2.437 mmol) was dissolved in anhydrous DMF (10 mL) and cooled to 0 °C. NaH (215 mg, 5.361 mmol) was slowly added, followed by dropwise addition of benzyl bromide (0.35 mL, 2.949 mmol), producing evanescence. The white suspension was stirred at room temperature, for 10 hours. Upon reaction completion, water (100 mL) was slowly added to the orange solution and the product was then extracted into ethyl acetate (3 x 40 mL). The combined organic layers were dried over MgSO_4 , filtered and concentrated under reduced pressure to afford a yellow oil. The oil was purified by flash column chromatography (DCM:methanol 85:15) to afford ether 9 as a white solid (502 mg, 70%). δ_{H} (500MHz, CDCl_3) 11.11 (s, 1H, *H*-7), 7.42 – 7.13 (m, 5H, *H*-11 + *H*-12 + *H*-13), 5.42 (d, $J = 8.3$ Hz, 1H, *H*-4), 4.53 (s, 2H, *H*-9), 4.51 – 4.42 (m, 1H, *H*-5), 3.81 (d, $J = 6.6$ Hz, 2H, *H*-8), 1.45 (s, 9H, *H*-1). δ_{C} (126MHz, CDCl_3) 175.5 (C=O), 155.8 (C=O), 137.3 (C-Ar), 128.5 (CH-Ar), 128.0 (CH-Ar), 127.9

(CH-Ar), 80.4 (C), 73.4 (CH₂), 69.7 (CH₂), 53.9 (CH), 28.3 (CH₃). **MS** (ES-) *m/z* 294.2 [M-H]⁻, 589.3 [2M-H]⁻. Analytical data is in agreement with literature values.⁷

***tert*-Butyl (S)-(3-(benzyloxy)-1-((4-hydroxyphenethyl)amino)-1-oxopropan-2-yl)carbamate (10).** To a stirring solution of compound **9** (502 mg, 1.699 mmol) in anhydrous THF (10 mL) was added triethylamine (0.43 mL, 3.048 mmol) and the resultant mixture stirred for five minutes. PyBOP (952 mg, 1.829 mmol) and tyramine (230 mg, 1.676 mmol) were then added and the solution was left to stir for 12 hours, at room temperature. The orange solution was concentrated to an orange oil and diluted with water (40 mL) and the product was extracted into ethyl acetate (3 x 40 mL). The combined orange organic layers were dried over MgSO₄, filtered and concentrated under reduced pressure to afford an orange oil. The oil was purified by flash column chromatography (ethyl acetate:hexane 7:3) to yield the desired product **10** as a white solid (670 mg, 95%). **δ_H** (500MHz, CDCl₃) 7.36 – 7.25 (m, 5H, *H*-9 + *H*-10 + *H*-11), 6.97 (d, *J* = 8.0 Hz, 2H, *H*-17), 6.73 (d, *J* = 8.1 Hz, 2H, *H*-18), 6.48 (s, 1H, *H*-13), 6.03 (s, 1H, *H*-4), 5.36 (bs, 1H, *H*-20), 4.48 (d, *J* = 11.7 Hz, 2H, *H*-7), 4.23 (s, 1H, *H*-5), 3.92 – 3.50 (m, 2H, *H*-6), 3.48 (t, *J* = 9.3 Hz, 2H, *H*-14), 2.69 (t, *J* = 6.9 Hz, 2H, *H*-15), 1.43 (s, 9H, *H*-1). **δ_C** (126MHz, CDCl₃) 170.3 (C=O), 154.6 (C=O), 154.6 (C-Ar), 137.4 (C-Ar), 130.4 (C-Ar), 129.8 (CH-Ar), 128.5 (CH-Ar), 128.0 (CH-Ar), 127.8 (CH-Ar), 115.5 (CH-Ar), 73.4 (CH₂), 69.9 (CH₂), 65.9 (C), 41.1 (CH₂), 34.7 (CH₂), 28.3 (CH₃), 28.1 (CH). **MS** (ES+) *m/z* 437.2 [M+Na]⁺.

(S)-2-Amino-3-hydroxy-*N*-(4-hydroxyphenethyl)propanamide (11). *Step One.* Synthesis of (S)-2-amino-3-(benzyloxy)-*N*-(4-hydroxyphenethyl)propanamide. Compound **10** (651 mg, 1.571 mmol) was dissolved in DCM (5 mL) and stirred with TFA (4 mL) at room temperature for three hours, producing evanescence. The dark red solution was then quenched with water (50 mL) and washed with DCM (1 x 20 mL), which was used to remove any residual impurities. The aqueous layer was then basified to pH 12 with 10% NaOH solution. The product was then extracted into ethyl acetate (3 x 30 mL) extraction. The colourless organic layers were combined, dried over MgSO₄, filtered and concentrated under reduced pressure to afford the desired amine as a white solid (480 mg, 97%). The product was used without purification. **δ_H** (500MHz, CD₃OD) 7.33 – 7.22 (m, 5H, *H*-15 + *H*-16 + *H*-17), 7.00 (d, *J* = 8.0 Hz, 2H, *H*-4), 6.72 (d, *J* = 8.0 Hz, 2H, *H*-3), 4.47 (s, 2H, *H*-13), 3.57 (d, *J* = 4.9 Hz, 2H, *H*-7), 3.54 – 3.46 (m, 1H, *H*-10), 3.44 – 3.32 (m, 2H, *H*-12), 2.67 (t, *J* = 7.1 Hz, 2H, *H*-6). **δ_C** (126MHz, CD₃OD) 170.2 (C=O), 155.7 (C-Ar), 137.0 (C-Ar), 129.7 (C-Ar), 129.4 (CH-Ar), 129.4 (CH-Ar), 128.2 (CH-Ar), 127.8 (CH-Ar), 114.9 (CH-Ar), 73.1 (CH₂), 67.9 (CH₂), 53.1 (CH), 41.0 (CH₂), 34.0 (CH₂). **MS** (ES+) *m/z* 651.3 [2M+Na]⁺.

Step Two. Synthesis of (S)-2-amino-3-hydroxy-*N*-(4-hydroxyphenethyl)propanamide (**11**). To a stirring solution of the intermediate amine (1.1 g, 3.499 mmol) in 1:1 mixture of anhydrous ethyl acetate (5 mL) and anhydrous ethanol (5 mL), was added a catalytic amount of Pd/C under a nitrogen atmosphere. The black suspension was degassed with nitrogen several times before being purged with hydrogen and being left to stir at room temperature, for one hour. The reaction mixture was filtered through a pad of Celite and washed with ethanol several times. Solvents were removed from the filtrate under reduced pressure to afford the product **11** as a white solid (770 mg, 99%), which was used without purification. **δ_H** (500MHz, DMSO) 9.30 (s, 1H, *H*-13), 8.59 (t, *J* = 5.2 Hz, 1H, *H*-6), 8.22 (s, 2H, *H*-1), 7.00 (d, *J* = 8.1 Hz, 2H, *H*-10), 6.70 (d, *J* = 8.2 Hz, 2H, *H*-11), 5.49 (s, 1H, *H*-4), 3.74 (dd, *J* = 15.8, 8.5 Hz, 2H, *H*-7), 3.69 – 3.60 (m, 1H, *H*-2), 3.35 – 3.17 (m, 2H, *H*-3), 2.62 (t, *J* = 7.2 Hz, 2H, *H*-8). **δ_C** (126MHz, DMSO) 167.0 (C=O), 156.3 (C-Ar), 130.0 (CH-Ar), 129.6 (C-Ar), 115.6 (CH-Ar), 60.8 (CH₂), 54.9 (CH), 41.3 (CH₂), 34.5 (CH₂). **MS** (ES+) *m/z* 449.3 [2M+H]⁺, 471.2 [2M+Na]⁺.

(S)-4-Azido-*N*-(2-((3-hydroxy-1-((4-hydroxyphenethyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)benzamide (12). Carboxylic acid **11** (100 mg, 0.454 mmol), triethylamine (0.32 mL, 2.270 mmol) and PyBOP (236 mg, 0.454 mmol) were dissolved in a solution of anhydrous DMF (4 mL) and then stirred for five minutes, at room temperature, under a nitrogen atmosphere. Compound **18** (102 mg, 0.454 mmol) was then added to the brown solution and stirred at room temperature, for 16 hours. The crude was diluted with water (50 mL) and the product was extracted into ethyl acetate (4 x 20 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated under reduced pressure to

afford a dark orange oil. Flash column chromatography (DCM:methanol 9:1) isolated the product **11** as a light cream solid (156 mg, 80%). δ_{H} (500 MHz, CD_3OD) 7.91 (d, $J = 8.8$ Hz, 2H, $H-2$), 7.16 (d, $J = 8.8$ Hz, 2H, $H-3$), 7.02 (d, $J = 8.6$ Hz, 2H, $H-17$), 6.69 (d, $J = 8.5$ Hz, 2H, $H-18$), 4.40 (t, $J = 5.2$ Hz, 1H, $H-9$), 4.06 (ABq, $J = 16.3$ Hz, 2H, $H-6$), 3.78 (td, $J = 11.1, 5.2$ Hz, 2H, $H-10$), 3.36 (dd, $J = 9.1, 6.6$ Hz, 2H, $H-14$), 2.70 (t, $J = 7.5$ Hz, 2H, $H-15$). δ_{C} (126MHz, CD_3OD) 170.7 (C=O), 170.6 (C=O), 168.3 (C=O), 155.5 (C-Ar), 143.9 (C-Ar), 130.0 (C-Ar), 129.8 (C-Ar), 129.4 (CH-Ar), 129.1 (CH-Ar), 118.6 (CH-Ar), 114.8 (CH-Ar), 61.5 (CH_2), 55.6 (CH), 43.1 (CH_2), 41.2 (CH_2), 34.2 (CH_2). **MS** (ES+) m/z 449.2 $[\text{M}+\text{Na}]^+$. **HRMS** (ESI) $\text{C}_{20}\text{H}_{23}\text{N}_6\text{O}_5$ calcd. 427.1730 $[\text{M}+\text{H}]^+$, found 427.1731. **HPLC** (reverse-phase) MeOH/ H_2O 0:100 to 100:0 in 12 min, $\lambda = 254$ nm, $t_{\text{R}} = 5.17$ min (96.0%). Analytical data is in agreement with literature values.⁸

(*S*)-2-(2-(4-Azidobenzamido)acetamido)-3-((4-hydroxyphenethyl)amino)-3-oxopropyl dihydrogen phosphate (13).

Step One. Synthesis of (*S*)-2-(2-(4-azidobenzamido)acetamido)-3-((4-hydroxyphenethyl)amino)-3-oxopropyl di(N,N-diisopropylphosphonamide). In a flame dried flask and under a nitrogen atmosphere, peptide **12** (144 mg, 0.338 mmol) was dissolved in a solution of anhydrous MeCN (7 mL) and anhydrous pyridine (3 mL). Tert-butyl magnesium chloride (0.75 mL, 0.743 mmol) and *N,N,N',N'*-tetramethylphosphorodiamidic chloride (0.05 mL, 0.338 mmol) was added and stirred for 24 h at room temperature. After reaction completion confirmed by TLC, the orange crude mixture was concentrated under reduced pressure and co-evaporated with methanol to azeotropically remove pyridine. The resulting orange oil was purified directly by flash column chromatography (DCM:methanol 95:5) to isolate the furnished diamidate as a cream solid (76 mg, 40%). δ_{H} (500MHz, CDCl_3) 8.29 (s, 1H, $H-6$), 7.82 (d, $J = 8.2$ Hz, 2H, $H-2$), 7.58 (d, $J = 5.4$ Hz, 1H, $H-9$), 7.26 (d, $J = 9.1$ Hz, 1H, $H-14$), 7.04 (d, $J = 8.1$ Hz, 2H, $H-3$), 6.99 – 6.90 (m, 4H, $H-18 + H-19$), 4.39 (s, 1H, $H-10$), 3.91 (d, $J = 12.0$ Hz, 2H, $H-7$), 3.72 – 3.66 (m, 2H, $H-11$), 3.36 (dd, $J = 11.9, 6.0$ Hz, 2H, $H-15$), 2.74 – 2.60 (m, 14H, $H-12 + H-16$). δ_{C} (126MHz, CDCl_3) 170.6 (C=O), 170.6 (C=O), 167.6 (C=O), 149.5 (C-Ar), 143.6 (C-Ar), 135.1 (C-Ar), 130.1 (CH-Ar), 129.5 (C-Ar), 129.4 (CH-Ar), 120.1 (CH-Ar), 118.8 (CH-Ar), 62.2 (CH_2), 55.0 (CH), 43.9 (CH_2), 40.6 (CH_2), 36.6 (CH_3), 34.3 (CH_2). δ_{P} (202MHz, CDCl_3) 10.53. **MS** (ES+) m/z 583.2 $[\text{M}+\text{Na}]^+$.

Step Two. Synthesis of (*S*)-2-(2-(4-azidobenzamido)acetamido)-3-((4-hydroxyphenethyl)amino)-3-oxopropyl dihydrogen phosphate (13). To a solution of diamidate intermediate from the previous step (67 mg, 0.120 mmol) in 1,4-dioxane (5 mL) was added 2N HCl solution (5 mL). The clear colourless/yellow solution was stirred at room temperature for 9 h. After TLC analysis confirmed total conversion, the mixture was concentrated under reduced pressure to afford the product **13** as a cream solid (55 mg, 91%). The product was then triturated with ethyl acetate (3 x 30 mL) and used without further purification. δ_{H} (500MHz, CD_3OD) δ 7.94 (d, $J = 8.7$ Hz, 2H, $H-2$), 7.21 – 7.03 (m, 6H, $H-3 + H-18 + H-19$), 4.39 (t, $J = 5.1$ Hz, 1H, $H-10$), 4.06 (ABq, $J = 16.4$ Hz, 2H, $H-7$), 3.78 (dd, $J = 11.2, 5.3$ Hz, 2H, $H-11$), 3.50 – 3.41 (m, 2H, $H-15$), 2.77 (t, $J = 6.2$ Hz, 2H, $H-16$). δ_{C} (126MHz, DMSO) 170.2 (C=O), 169.5 (C=O), 166.2 (C=O), 151.3 (C-Ar), 142.9 (C-Ar), 134.4 (C-Ar), 131.0 (C-Ar), 129.8 (CH-Ar), 129.8 (CH-Ar), 121.4 (CH-Ar), 119.4 (CH-Ar), 62.0 (CH_2), 55.8 (CH), 43.3 (CH_2), 41.0 (CH_2), 34.8 (CH_2). δ_{P} (202MHz, CD_3OD) -5.14. **MS** (ES+) m/z 507.1 $[\text{M}+\text{H}]^+$. **HRMS** (ESI) $\text{C}_{20}\text{H}_{24}\text{N}_6\text{O}_8\text{P}$ calcd. 507.1393 $[\text{M}+\text{H}]^+$, found 507.1396. **HPLC** (reverse-phase) MeOH/ H_2O 0:100 to 100:0 in 12 min, $\lambda = 254$ nm, $t_{\text{R}} = 5.66$ min (87.6%).

(*S*)-2-(2-(4-Azidobenzamido)acetamido)-3-((4-hydroxyphenethyl)amino)-3-oxopropyl diethyl phosphate (14). In a flame dried flask and under a nitrogen atmosphere, peptide **12** (60 mg, 0.141 mmol) was dissolved in a solution of anhydrous MeCN (7 mL) and anhydrous pyridine (3 mL). Tert-butyl magnesium chloride (0.28 mL, 0.282 mmol) and diethyl chlorophosphate (0.02 mL, 0.155 mmol) was added and stirred for 24 h at room temperature. After reaction completion confirmed by TLC, the orange crude mixture was concentrated under reduced pressure and co-evaporated with methanol to azeotropically remove pyridine. The resulting orange oil was purified directly by flash column chromatography (DCM:methanol 95:5) to isolate the furnished compound **14** as a colourless oil (24 mg,

30%). δ_{H} (500MHz, CDCl_3) 7.92 (t, $J = 5.2$ Hz, 1H, $H-6$), 7.86 (d, $J = 8.6$ Hz, 2H, $H-2$), 7.32 (d, $J = 7.6$ Hz, 1H, $H-9$), 7.11 (d, $J = 8.5$ Hz, 2H, $H-3$), 7.08 – 7.00 (m, 4H, $H-19 + H-20$), 6.93 (t, $J = 5.7$ Hz, 1H, $H-15$), 4.39 (dt, $J = 7.8, 4.0$ Hz, 1H, $H-10$), 4.28 – 4.16 (m, 4H, $H-12$), 4.10 – 3.82 (m, 3H, $H-7 + H-11a/b$), 3.60 (dd, $J = 11.3, 4.7$ Hz, 1H, $H-11a/b$), 3.57 – 3.39 (m, 2H, $H-16$), 2.84 – 2.79 (m, 2H, $H-17$), 1.38 (dd, $J = 7.5, 6.6$ Hz, 6H, $H-13$). δ_{C} (126MHz, CDCl_3) 170.7 (C=O), 170.1 (C=O), 167.5 (C=O), 149.1 (C-Ar), 143.8 (C-Ar), 135.7 (C-Ar), 130.2 (CH-Ar), 129.7 (C-Ar), 129.3 (CH-Ar), 120.1 (CH-Ar), 118.9 (CH-Ar), 64.8 (CH_2), 62.1 (CH_2), 54.2 (CH), 44.1 (CH_2), 40.2 (CH_2), 34.3 (CH_2), 16.1 (CH_3). δ_{P} (202MHz, CDCl_3) -6.11. **MS** (ES+) m/z 585.2 $[\text{M}+\text{Na}]^+$. **HRMS** (ESI) $\text{C}_{24}\text{H}_{32}\text{N}_6\text{O}_8\text{P}$ calcd. 563.2019 $[\text{M}+\text{H}]^+$, found 563.2034. **HPLC** (reverse-phase) MeOH/ H_2O 0:100 to 100:0 in 12 min, $\lambda = 254$ nm, $t_{\text{R}} = 5.87$ min (96%).

Methyl (((S)-2-(2-(4-azidobenzamido)acetamido)-3-((4-hydroxyphenethyl)amino)-3-oxopropoxy)(phenoxy)phosphoryl)-L-alaninate (15a). In a flame dried flask and under a nitrogen atmosphere, peptide **12** (50 mg, 0.117 mmol) was dissolved in a solution of anhydrous MeCN (7 mL) and anhydrous pyridine (3 mL). *tert*-Butyl magnesium chloride ($t\text{-BuMgCl}$) (0.26 mL, 0.257 mmol) and chloridate **7** (36 mg, 0.129 mmol) was added and stirred for 24 h at room temperature. After reaction completion confirmed by TLC, the orange crude mixture was concentrated under reduced pressure and co-evaporated with methanol to azeotropically remove pyridine. The resulting orange oil was purified directly by flash column chromatography (DCM:methanol 95:5) to isolate the desired prodrug **15a** as a cream solid (23 mg, 29%). δ_{H} (500MHz, CDCl_3) 8.01 – 7.91 (m, 1H, $H-6$), 7.80 (dd, $J = 11.2, 6.3$ Hz, 2H, $H-2$), 7.46 (dd, $J = 11.3, 5.9$ Hz, 1H, $H-9$), 7.33 (dd, $J = 11.2, 7.5$ Hz, 2H, $H-14$), 7.28 – 7.19 (m, 3H, $H-13 + H-15$), 7.13 – 6.97 (m, 5H, $H-22 + H-26 + H-27$), 6.94 (dd, $J = 8.6, 3.7$ Hz, 2H, $H-3$), 4.52 – 4.29 (m, 2H, $H-10 + H-16$), 4.15 – 3.80 (m, 4H, $H-7 + H-11a/b + H-17$), 3.68 (d, $J = 7.5$ Hz, 3H, $H-20$), 3.63 – 3.55 (m, 1H, $H-11a/b$), 3.46 – 3.36 (m, 2H, $H-23$), 2.75 (t, $J = 6.3$ Hz, 2H, $H-24$), 1.39 (d, $J = 3.1$ Hz, 3H, $H-18$). δ_{C} (126MHz, CDCl_3) 176.6 (C=O), 170.7 (C=O), 170.0 (C=O), 167.4 (C=O), 150.4 (C-Ar), 148.9 (C-Ar), 143.7 (C-Ar), 135.8 (C-Ar), 130.2 (CH-Ar), 129.8 (CH-Ar), 129.7 (C-Ar), 129.3 (CH-Ar), 125.4 (CH-Ar), 120.3 (CH-Ar), 120.2 (CH-Ar), 118.9 (CH-Ar), 62.2 (CH_2), 54.2 (CH), 52.6 (CH_3), 50.4 (CH), 43.9 (CH_2), 40.1 (CH_2), 34.3 (CH_2), 20.8 (CH_3). δ_{P} (202MHz, CDCl_3) -2.07, -2.13. **MS** (ES+) m/z 690.2 $[\text{M}+\text{Na}]^+$. **HRMS** (ESI) $\text{C}_{30}\text{H}_{35}\text{N}_7\text{O}_9\text{P}$ calcd. 668.2234 $[\text{M}+\text{H}]^+$, found 668.2230. **HPLC** (reverse-phase) MeOH/ H_2O 0:100 to 100:0 in 12 min, $\lambda = 254$ nm, $t_{\text{R}} = 6.20$ min (96%).

Isopropyl (((S)-2-(2-(4-azidobenzamido)acetamido)-3-((4-hydroxyphenethyl)amino)-3-oxopropoxy)(phenoxy)phosphoryl)-L-alaninate (15b). In a flame dried flask and under a nitrogen atmosphere, peptide **12** (50 mg, 0.117 mmol) was dissolved in a solution of anhydrous MeCN (7 mL) and anhydrous pyridine (3 mL). *t*-BuMgCl (0.26 mL, 0.257 mmol) and chloridate **20a** (40 mg, 0.129 mmol) was added and stirred for 24 h at room temperature. After reaction completion confirmed by TLC, the orange crude mixture was concentrated under reduced pressure and co-evaporated with methanol to azeotropically remove pyridine. The resulting orange oil was purified directly by flash column chromatography (DCM:methanol 95:5) to isolate the furnished prodrug **15b** as a cream solid (22 mg, 27%). δ_{H} (500MHz, CDCl_3) 7.97 (dd, $J = 10.2, 4.4$ Hz, 1H, $H-6$), 7.81 (dd, $J = 8.5, 2.4$ Hz, 2H, $H-2$), 7.45 (dd, $J = 12.9, 6.3$ Hz, 1H, $H-9$), 7.36 – 7.30 (m, 2H, $H-14$), 7.25 – 7.14 (m, 3H, $H-13 + H-15$), 7.13 – 6.99 (m, 5H, $H-23 + H-27 + H-28$), 6.93 (dd, $J = 8.6, 3.6$ Hz, 2H, $H-3$), 5.09 – 5.00 (m, 1H, $H-20$), 4.46 – 4.24 (m, 2H, $H-10 + H-16$), 4.08 – 3.79 (m, 4H, $H-7 + H-11a/b + H-17$), 3.59 (d, $J = 6.4$ Hz, 1H, $H-11a/b$), 3.41 (dt, $J = 11.2, 6.4$ Hz, 2H, $H-24$), 2.80 – 2.75 (m, 2H, $H-25$), 1.38 (dd, $J = 6.9, 3.1$ Hz, 3H, $H-18$), 1.22 (dd, $J = 5.3, 3.3$ Hz, 6H, $H-21$). δ_{C} (126MHz, CDCl_3) 172.8 (C=O), 170.6 (C=O), 170.1 (C=O), 167.4 (C=O), 150.5 (C-Ar), 149.0 (C-Ar), 143.6 (C-Ar), 135.8 (C-Ar), 130.2 (CH-Ar), 129.8 (CH-Ar), 129.7 (C-Ar), 129.3 (CH-Ar), 125.3 (CH-Ar), 120.3 (CH-Ar), 120.2 (CH-Ar), 118.9 (CH-Ar), 69.4 (CH), 62.2 (CH_2), 54.5 (CH), 50.5 (CH), 43.8 (CH_2), 40.3 (CH_2), 34.3 (CH_2), 21.6 (CH_3), 20.9 (CH_3). δ_{P} (202MHz, CDCl_3) -1.93, -2.03. **MS** (ES+) m/z 718.2 $[\text{M}+\text{Na}]^+$. **HRMS** (ESI) $\text{C}_{32}\text{H}_{39}\text{N}_7\text{O}_9\text{P}$ calcd. 696.2547 $[\text{M}+\text{H}]^+$, found 696.2541. **HPLC** (reverse-phase) MeOH/ H_2O 0:100 to 100:0 in 12 min, $\lambda = 254$ nm, $t_{\text{R}} = 7.53$ min (96.0%).

Benzyl (((S)-2-(2-(4-azidobenzamido)acetamido)-3-((4-hydroxyphenethyl)amino)-3-oxopropoxy)(phenoxy)phosphoryl)-D-alaninate (15c). In a flame dried flask and under a nitrogen atmosphere, peptide **12** (160 mg, 0.375 mmol) was dissolved in a solution of anhydrous MeCN (7 mL) and anhydrous pyridine (3 mL). ^tBuMgCl (0.83 mL, 0.825 mmol) and chloridate **20b** (133 mg, 0.375 mmol) was added and stirred for 24 h at room temperature. After reaction completion confirmed by TLC, the orange crude mixture was concentrated under reduced pressure and co-evaporated with methanol to azeotropically remove pyridine. The resulting orange oil was purified directly by flash column chromatography (DCM:methanol 95:5) to isolate the desired prodrug **15c** as a cream solid (58 mg, 29%). δ_{H} (500MHz, CDCl₃) 7.90 (dd, *J* = 15.5, 5.0 Hz, 1H, *H*-6), 7.81 (dd, *J* = 9.6, 2.8 Hz, 2H, *H*-2), 7.43 – 7.34 (m, 1H, *H*-9), 7.35 – 7.25 (m, 7H, *H*-14 + *H*-22 + *H*-23 + *H*-24), 7.24 – 7.13 (m, 3H, *H*-13 + *H*-15), 7.08 – 6.97 (m, 5H, *H*-26 + *H*-30 + *H*-31), 6.93 (dd, *J* = 8.6, 4.4 Hz, 2H, *H*-3), 5.12 (dd, *J* = 5.9, 2.0 Hz, 2H, *H*-20), 4.53 – 4.28 (m, 2H, *H*-10 + *H*-16), 4.12 (dd, *J* = 9.2, 4.9 Hz, 1H, *H*-17), 4.04 – 3.76 (m, 3H, *H*-7 + *H*-11a/b), 3.62 – 3.54 (m, 1H, *H*-11a/b), 3.46 – 3.40 (m, 2H, *H*-27), 2.84 – 2.64 (m, 2H, *H*-28), 1.40 (dd, *J* = 6.7, 2.4 Hz, 3H, *H*-18). δ_{C} (126MHz, CDCl₃) 173.1 (C=O), 170.6 (C=O), 170.0 (C=O), 167.4 (C=O), 150.4 (C-Ar), 148.9 (C-Ar), 143.6 (C-Ar), 135.8 (C-Ar), 135.1 (C-Ar), 130.2 (CH-Ar), 129.8 (CH-Ar), 129.7, (C-Ar) 129.3 (CH-Ar), 128.7 (CH-Ar), 128.6 (CH-Ar), 128.2 (CH-Ar), 125.4 (CH-Ar), 120.3 (CH-Ar), 120.2 (CH-Ar), 118.9 (CH-Ar), 67.4 (CH₂), 62.2 (CH₂), 54.4 (CH), 50.5 (CH), 43.8 (CH₂), 40.3 (CH₂), 34.3 (CH₂), 20.8 (CH₃). δ_{P} (202MHz, CDCl₃) -2.03, -2.13. **MS** (ES+) *m/z* 766.2 [M+Na]⁺. **HRMS** (ESI) C₃₆H₃₉N₇O₉P calcd. 744.2547 [M+H]⁺, found 744.2548. **HPLC** (reverse-phase) MeOH/H₂O 0:100 to 100:0 in 12 min, λ = 254 nm, *t_R* = 8.64 min (95.3%).

Ethyl (4-azidobenzoyl)glycinate (17). 4-Azidobenzoic acid **16** (322 mg, 2.000 mmol) was dissolved in anhydrous DCM (20 mL) and stirred with DIPEA (0.77 mL, 4.400 mmol) for five minutes at room temperature. PyBOP (1.145 g, 2.200 mmol) and glycine ethyl ester hydrochloride (307 mg, 2.200 mmol) was added to the colourless solution at 0 °C and stirred at room temperature for 3 h. Water (20 mL) was then poured into the yellow solution and the product was extracted into DCM washes (2 x 20 mL), dried over MgSO₄, filtered and concentrated under reduced pressure to afford an orange oil. The oil was purified via flash column chromatography (ethyl acetate:hexane 1:1) to yield the amide **17** as a white solid (455 mg, 92%). δ_{H} (500MHz, CDCl₃) 7.82 (d, *J* = 8.2 Hz, 2H, *H*-3), 7.08 (d, *J* = 8.2 Hz, 2H, *H*-2), 6.60 (s, 1H, *H*-6), 4.27 (q, *J* = 7.1 Hz, 2H, *H*-9), 4.23 (d, *J* = 4.8 Hz, 2H, *H*-7), 1.32 (t, *J* = 7.1 Hz, 3H, *H*-10). δ_{C} (126MHz, CDCl₃) 170.1 (C=O), 166.3 (C=O), 143.7 (C-Ar), 130.2 (C-Ar), 128.9 (CH-Ar), 119.1 (CH-Ar), 61.8 (CH₂), 41.9 (CH₂), 14.2 (CH₃). **MS** (ES+) *m/z* 271.1 [M+Na]⁺, 519.2 [2M+Na]⁺. Analytical data is in agreement with literature values.⁹

(4-Azidobenzoyl)glycine (18). To a stirring solution of azide **17** (300 mg, 1.218 mmol) in THF (8 mL), 10% NaOH (30 mg, 1.218 mmol) was added and stirred at room temperature for 1 h. After TLC confirmed reaction completion, the orange solution was concentrated under reduced pressure and diluted with water (20 mL) and carefully acidified to pH with 6N HCl, forming a white suspension. The product was extracted out of the suspension with ethyl acetate (3 x 20 mL), dried under MgSO₄, filtered and concentrated under reduced pressure to yield the desired acid **18** as a white solid (263 mg, 98%).

δ_{H} (500MHz, CD₃OD) 7.90 (d, *J* = 8.2 Hz, 2H, *H*-3), 7.16 (d, *J* = 8.3 Hz, 2H, *H*-2), 4.08 (d, *J* = 4.5 Hz, 2H, *H*-7). δ_{C} (126MHz, CD₃OD) 171.7 (C=O), 168.0 (C=O), 143.7 (C-Ar), 130.2 (C-Ar), 128.9 (CH-Ar), 118.6 (CH-Ar), 40.9 (CH₂). **MS** (ES-) *m/z* 215.1 [M-H]⁻. Analytical data is in agreement with literature values.⁹

Isopropyl (chloro(phenoxy)phosphoryl)-L-alaninate (20a). To a stirring solution of compound **5** (0.30 mL, 1.969 mmol) and anhydrous DCM (10 mL), in a flame dried flask, was added *L*-alanine isopropyl ester hydrochloride (300 mg, 1.790 mmol) and stirred for 15 minutes at room temperature, under a nitrogen atmosphere. Anhydrous triethylamine (0.51 mL, 3.580 mmol) was added dropwise to the yellow solution at -78 °C to form a suspended white precipitate. The reaction mixture was stirred for 30 minutes at -78 °C before being left to stir and warm up to room temperature over 2.5 hours. Upon reaction completion, the colourless solution was concentrated under reduced pressure, and then diethyl ether was

added to the obtained material. After gentle mixing and filtration, the yellow filtrate was concentrated under reduced pressure to afford a colourless oil, which was purified with column chromatography (ethyl acetate:hexane 6:4) to yield compound **20a** as a yellow oil (463 mg, 86%). δ_{H} (500MHz, CDCl_3) 7.38 (t, $J = 9.0$ Hz, 2H, $H-3$), 7.29 – 7.18 (m, 3H, $H-2 + H-4$), 5.19 – 5.00 (m, 1H, $H-9$), 4.36 – 3.94 (m, 2H, $H-5 + H-6$), 1.49 (d, $J = 2.3$ Hz, 3H, $H-7$), 1.27 (dd, $J = 8.0, 6.2$ Hz, 6H, $H-10$). δ_{P} (202MHz, CDCl_3) 8.04, 7.65. Analytical data is in agreement with literature values.¹⁰

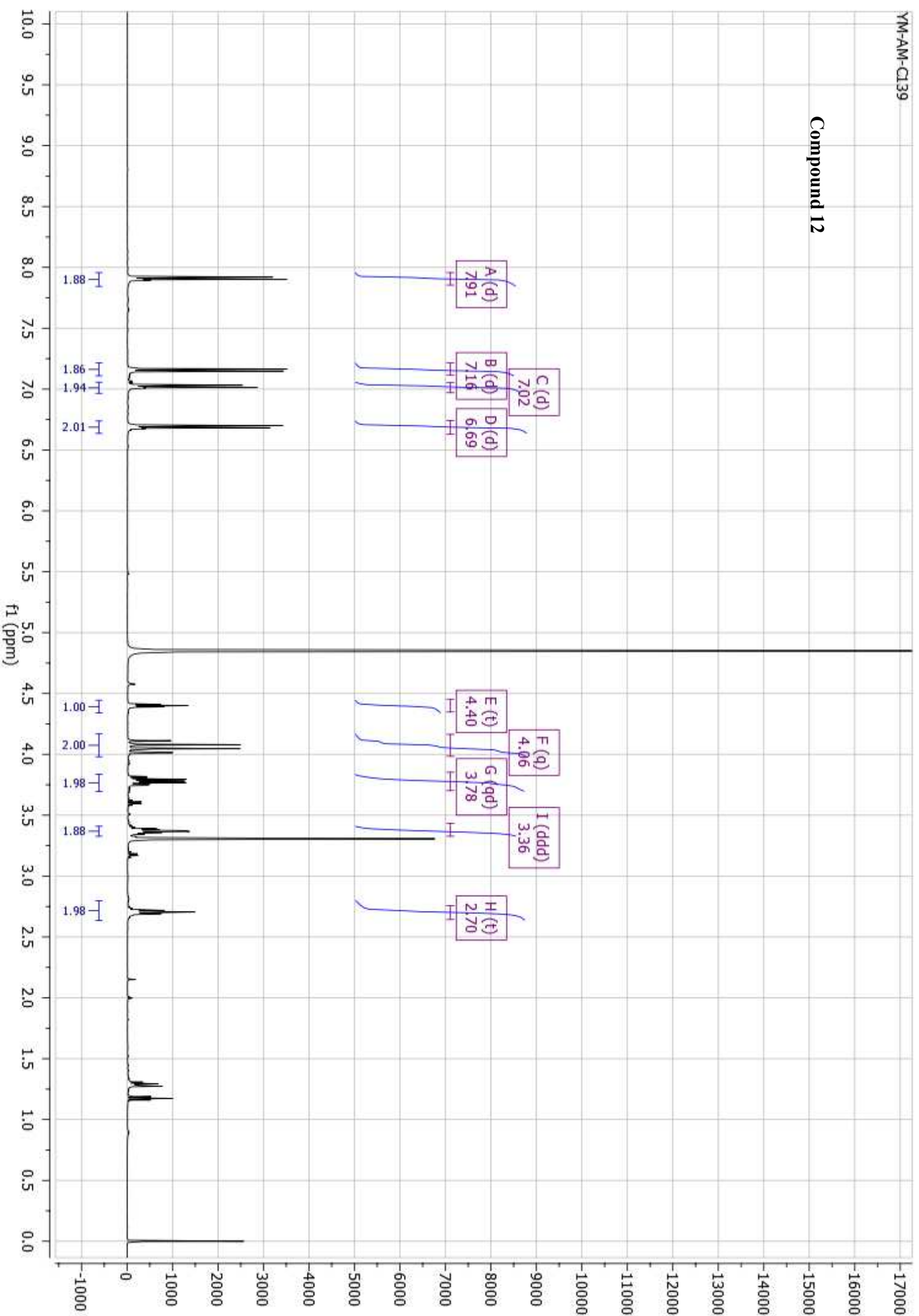
Benzyl (chloro(phenoxy)phosphoryl)-L-alaninate (20b). To a stirring solution of compound **5** (0.23 mL, 1.530 mmol) and anhydrous DCM (10 mL), in a flame dried flask, was added L-alanine benzyl ester hydrochloride (300 mg, 1.391 mmol) and stirred for 15 minutes at room temperature, under a nitrogen atmosphere. Anhydrous triethylamine (0.39 mL, 2.782 mmol) was added dropwise to the yellow solution at -78°C to form a suspended white precipitate. The reaction mixture was stirred for 30 minutes at -78°C before being left to stir and warm up to room temperature over 2.5 hours. Upon reaction completion, the colourless solution was concentrated under reduced pressure, and then diethyl ether was added to the obtained material. After gentle mixing and filtration, the yellow filtrate was concentrated under reduced pressure to afford a colourless oil, which was purified with column chromatography (ethyl acetate:hexane 6:4) to yield compound **20b** as a yellow oil (410 mg, 83%). δ_{H} (500MHz, CDCl_3) 7.45 – 7.28 (m, 7H, $H-3 + H-11 + H-12 + H-13$), 7.28 – 7.21 (m, 3H, $H-2 + H-4$), 5.21 (d, $J = 7.7$ Hz, 2H, $H-9$), 4.36 – 4.12 (m, 2H, $H-5 + H-6$), 1.52 (dd, $J = 6.8, 3.0$ Hz, 3H, $H-7$). δ_{P} (202MHz, CDCl_3) 7.84, 7.49. Analytical data is in agreement with literature values.¹⁰

REFERENCES

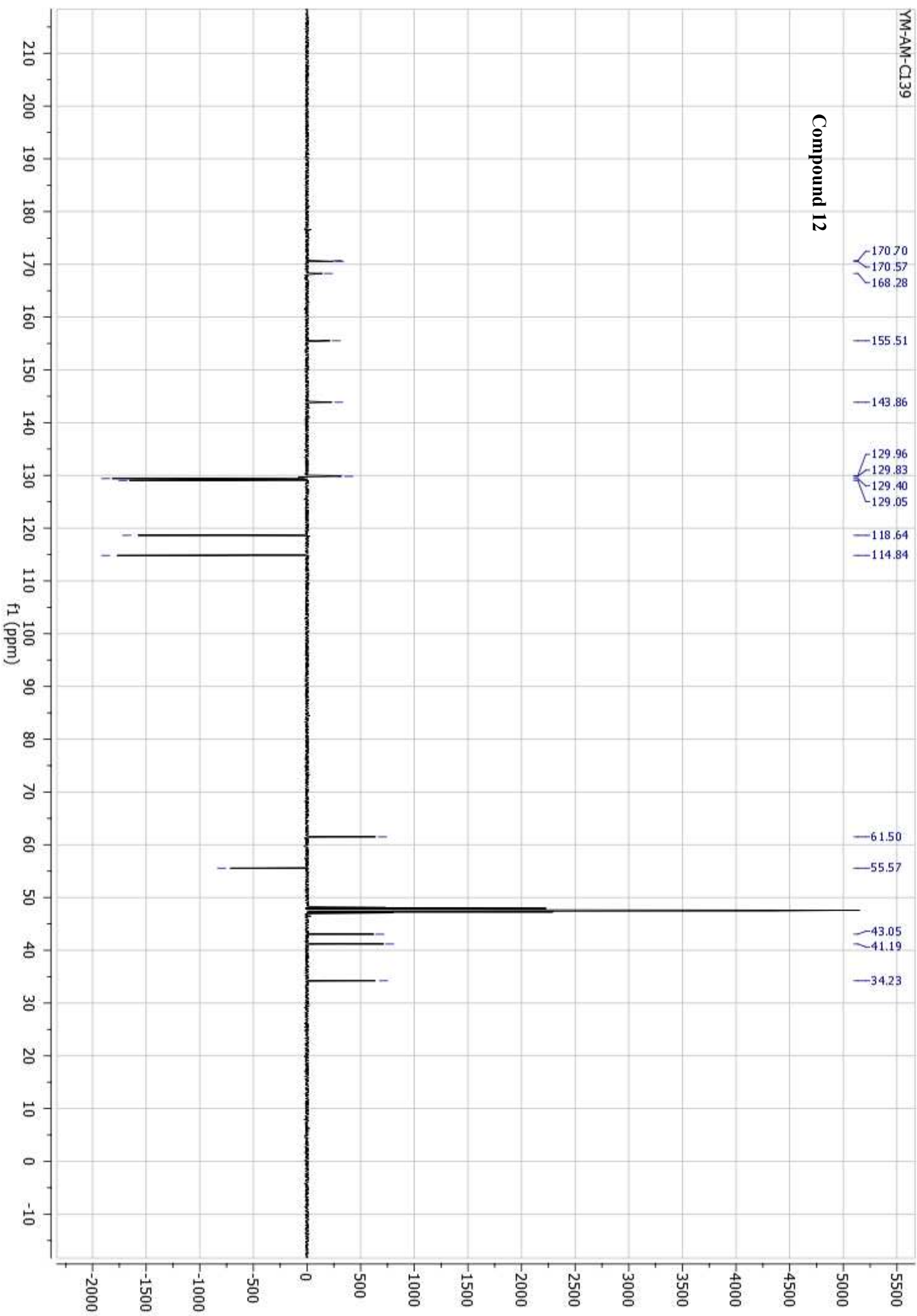
1. Mehellou, Y.; Valente, R.; Mottram, H.; Walsby, E.; Mills, K. I.; Balzarini, J.; McGuigan, C. Phosphoramidates of 2'-beta-D-arabinouridine (AraU) as phosphate prodrugs; design, synthesis, in vitro activity and metabolism. *Bioorg. Med. Chem.* **2010**, 18, 2439-46.
2. Lima, C. D.; Klein, M. G.; Hendrickson, W. A. Structure-based analysis of catalysis and substrate definition in the HIT protein family. *Science* **1997**, 278, 286-90.
3. Hawkins, P. C.; Nicholls, A. Conformer generation with OMEGA: learning from the data set and the analysis of failures. *J. Chem. Inf. Model.* **2012**, 52, 2919-36.
4. McGann, M. FRED pose prediction and virtual screening accuracy. *J. Chem. Inf. Model.* **2011**, 51, 578-96.
5. McGann, M. R.; Almond, H. R.; Nicholls, A.; Grant, J. A.; Brown, F. K. Gaussian docking functions. *Biopolymers* **2003**, 68, 76-90.
6. Meneghesso, S.; Vanderlinden, E.; Brancale, A.; Balzarini, J.; Naesens, L.; McGuigan, C. Synthesis and biological evaluation of purine 2'-fluoro-2'-deoxyriboside ProTides as anti-influenza virus agents. *ChemMedChem* **2013**, 8, 415-25.
7. Qi, X. L.; Yang, E. Q.; Zhang, J. T.; Wang, T.; Cao, X. P. A Facile Synthesis of the Spiroindoline-Based Growth Hormone Secretagogue, MK-677. *Chinese Chem. Lett.* **2012**, 23, 661-664.
8. Wu, H.; Ge, J.; Yao, S. Q. Microarray-assisted high-throughput identification of a cell-permeable small-molecule binder of 14-3-3 proteins. *Angew. Chem. Int. Ed. Engl.* **2010**, 49, 6528-32.
9. Arrendale, A.; Kim, K.; Choi, J. Y.; Li, W.; Geahlen, R. L.; Borch, R. F. Synthesis of a phosphoserine mimetic prodrug with potent 14-3-3 protein inhibitory activity. *Chem. Biol.* **2012**, 19, 764-71.
10. Osgerby, L.; Lai, Y. C.; Thornton, P. J.; Amalfitano, J.; Le Duff, C. S.; Jabeen, I.; Kadri, H.; Miccoli, A.; Tucker, J. H. R.; Muqit, M. M. K.; Mehellou, Y. Kinetin Riboside and Its ProTides Activate the Parkinson's Disease Associated PTEN-Induced Putative Kinase 1 (PINK1) Independent of Mitochondrial Depolarization. *J. Med. Chem.* **2017**, 60, 3518-3524.

NMR SPECTRA AND HPLC DATA

Compound 12



Compound 12





SHIMADZU

LabSolutions

Analysis Report

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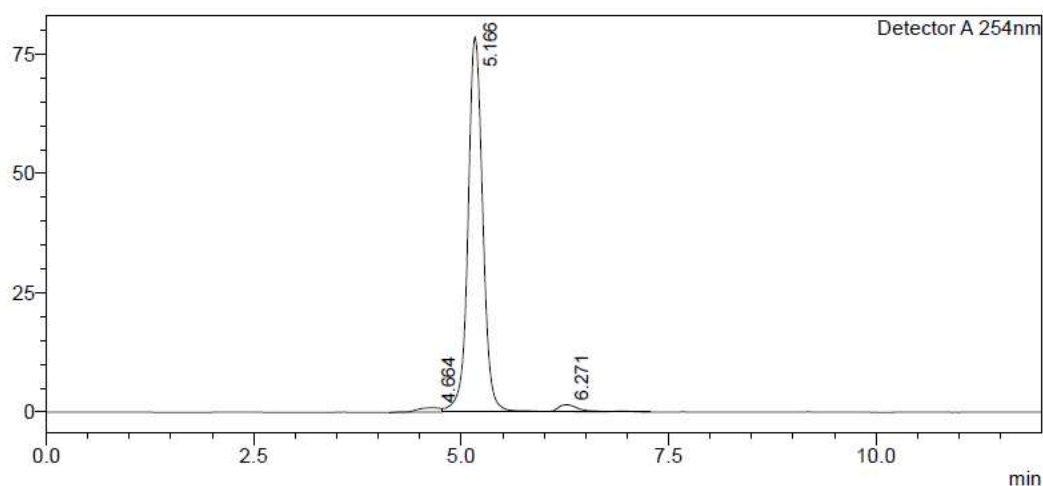
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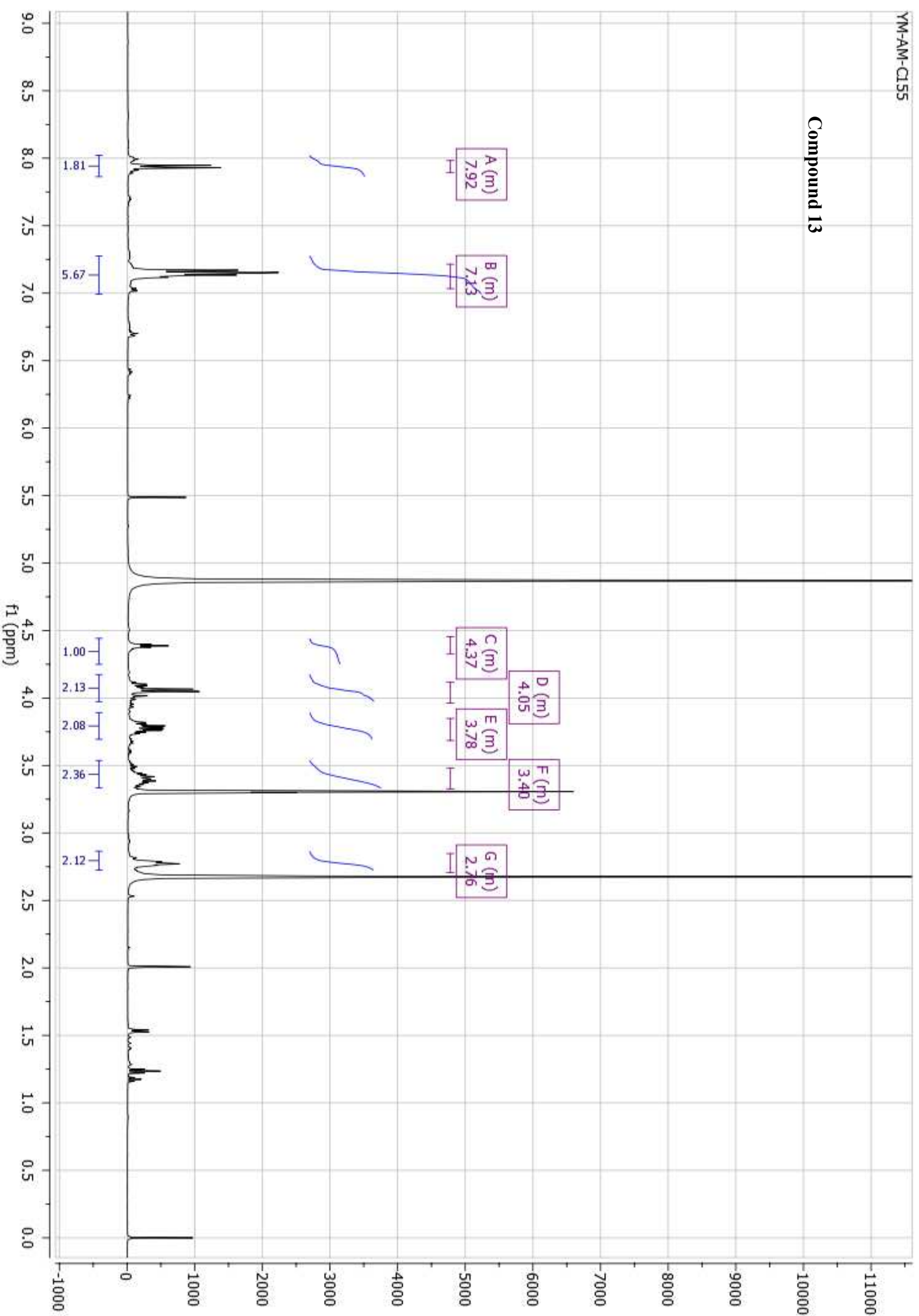
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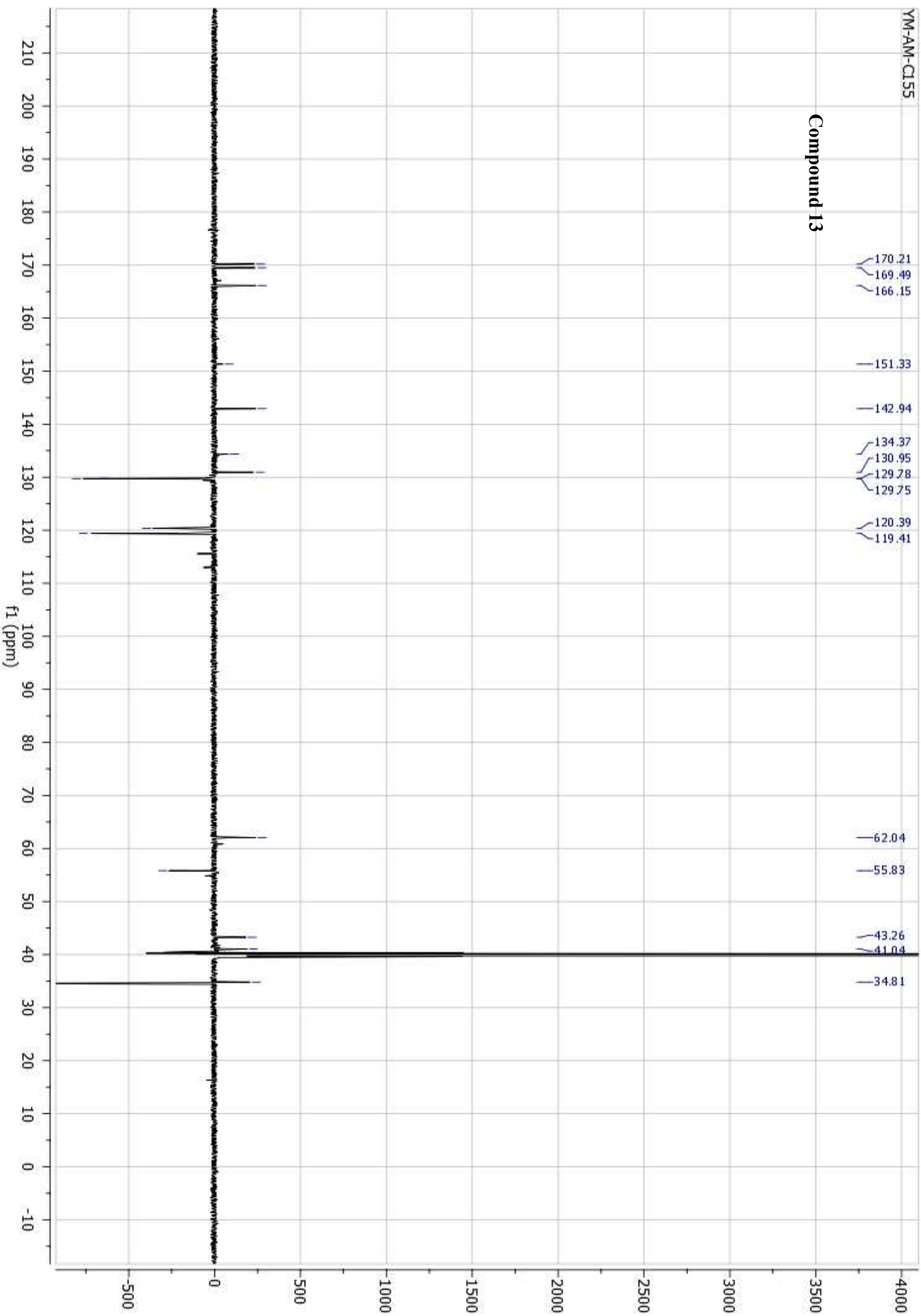
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Compound 12

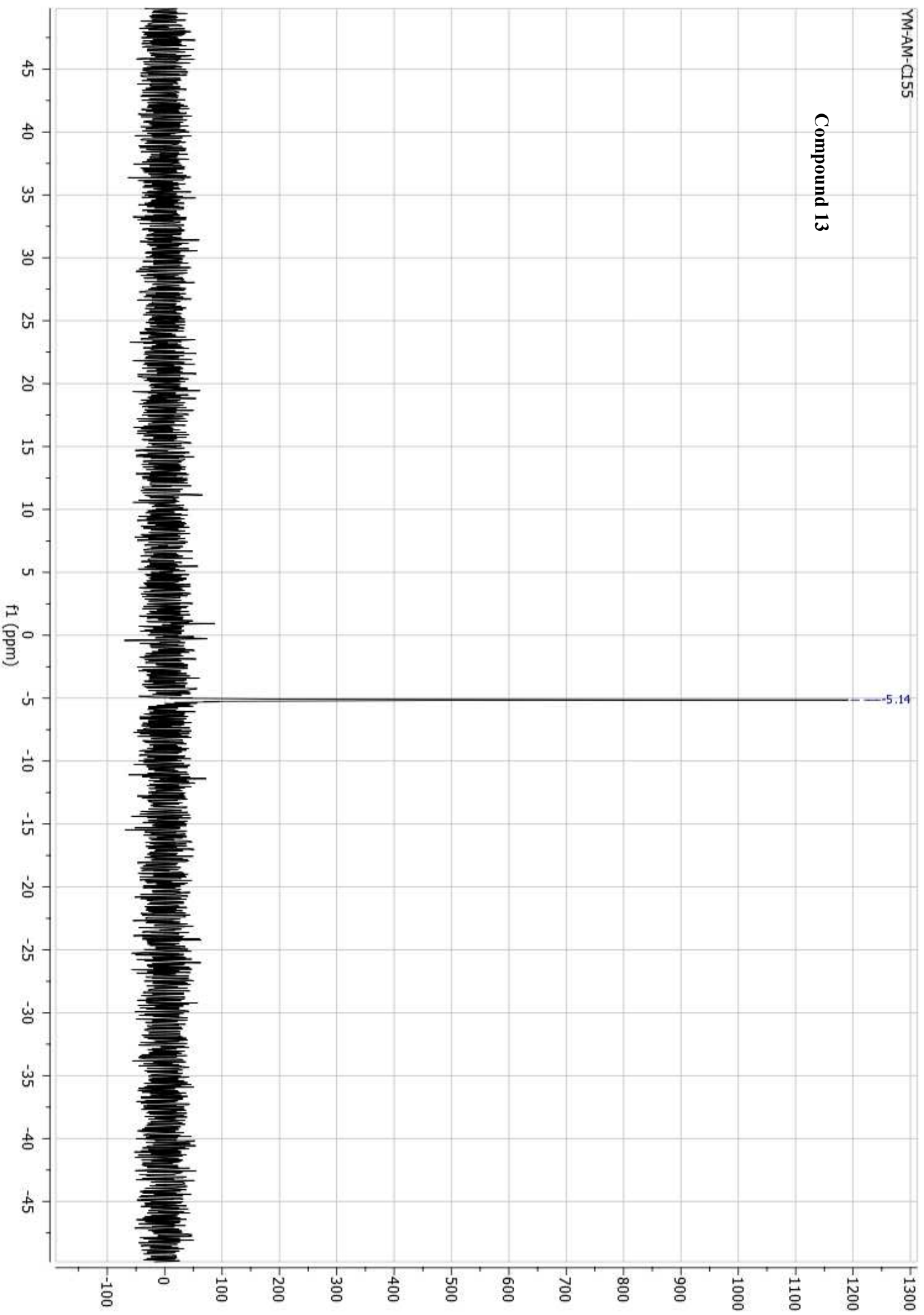
Compound 13



Compound 13



Compound 13





SHIMADZU
LabSolutions

Analysis Report

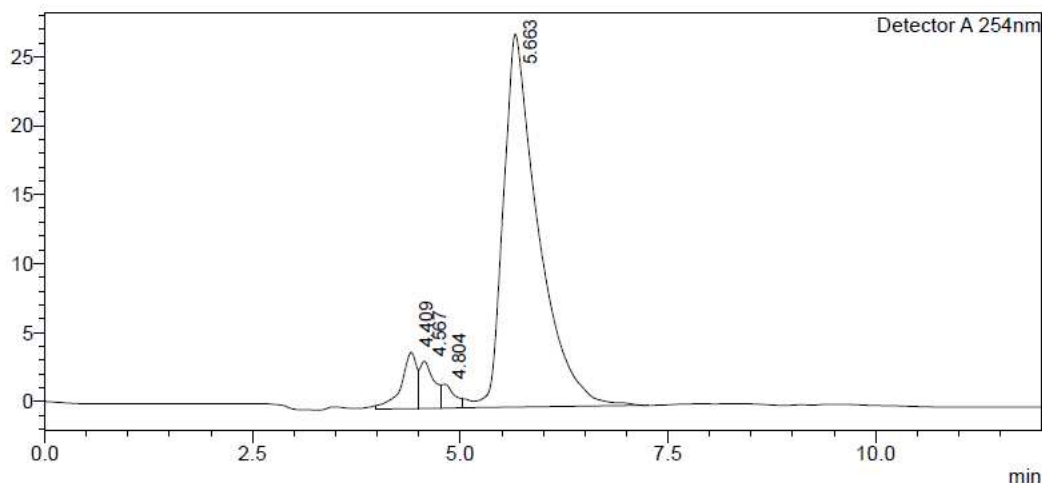
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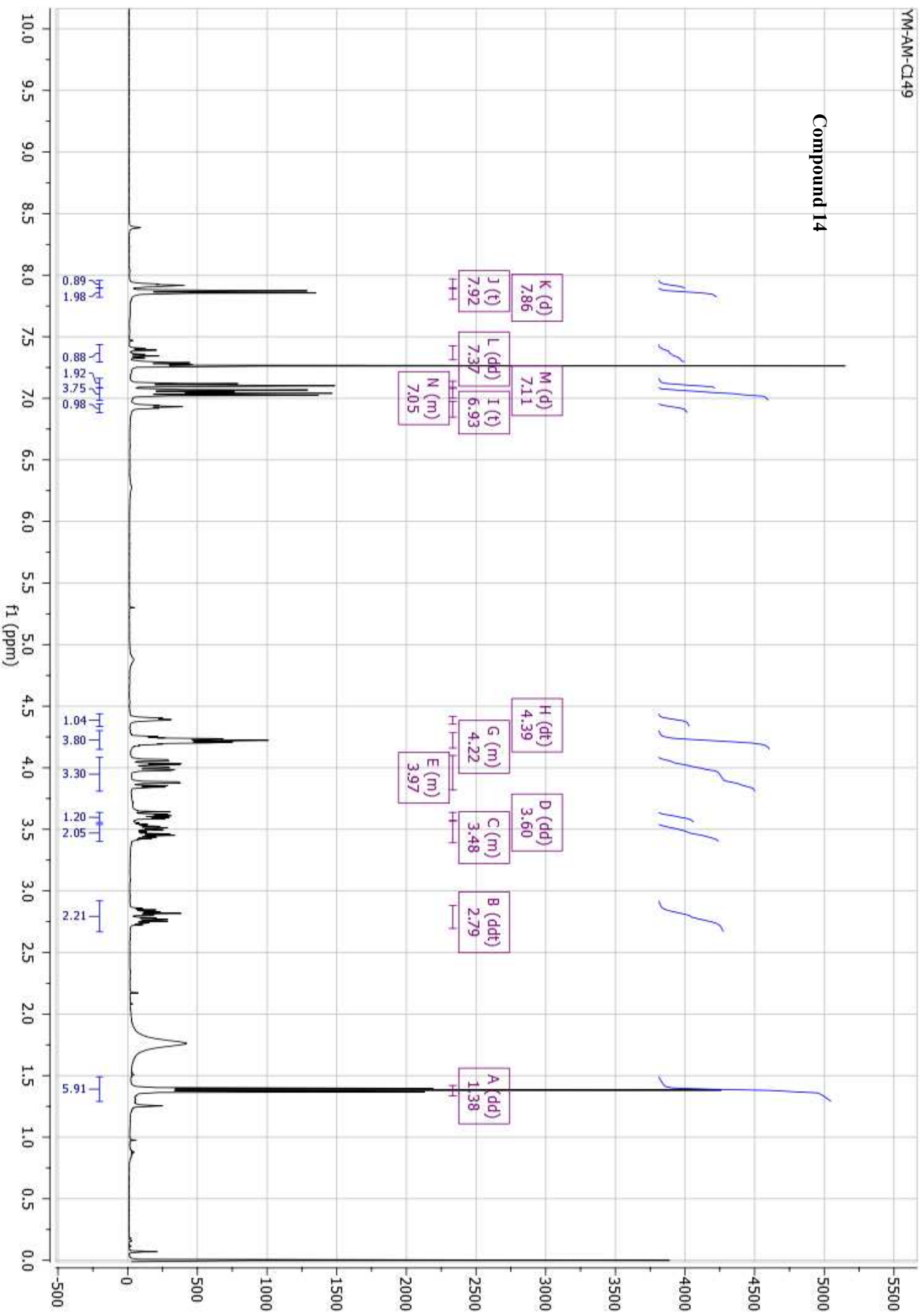
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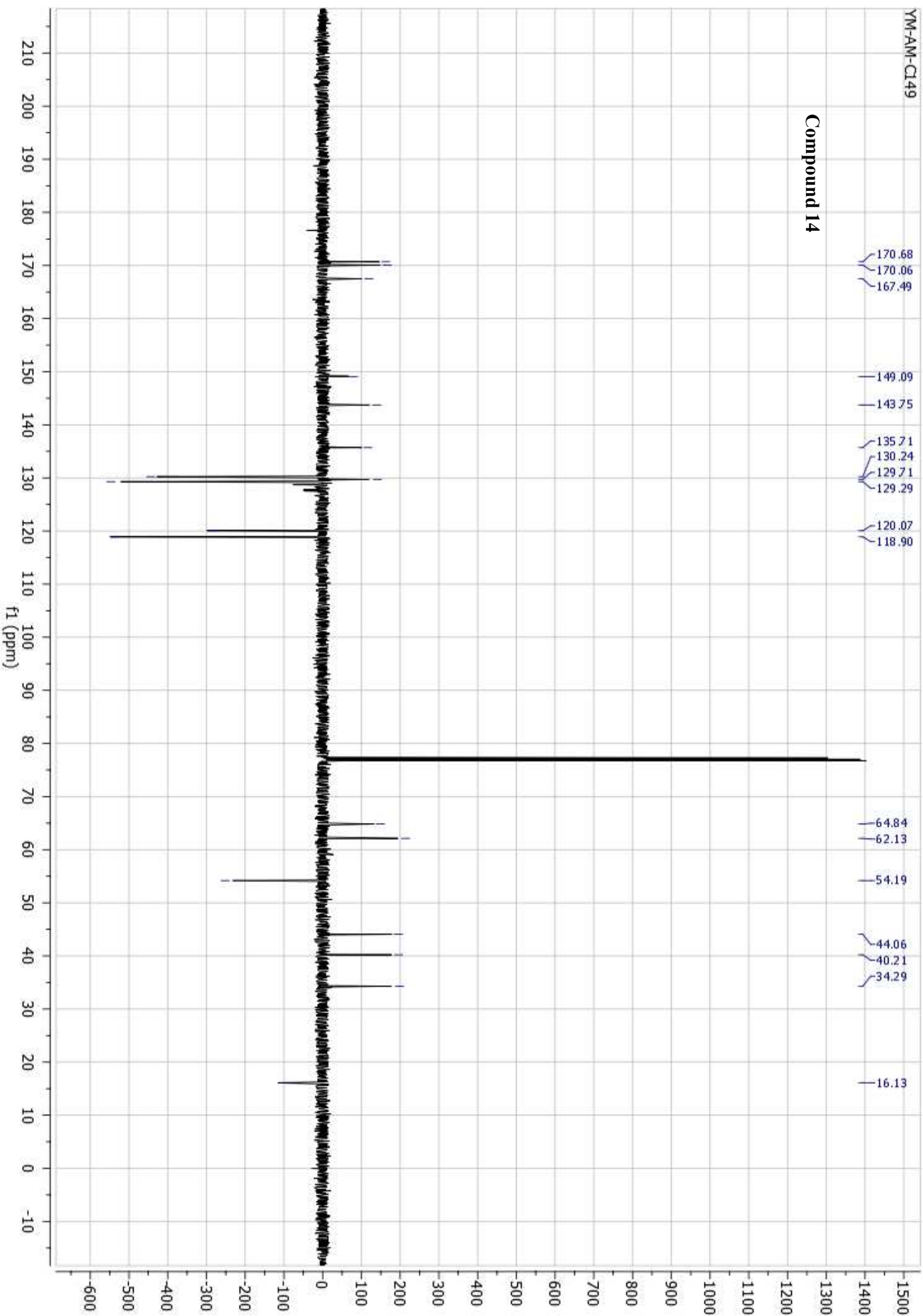
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Compound 13

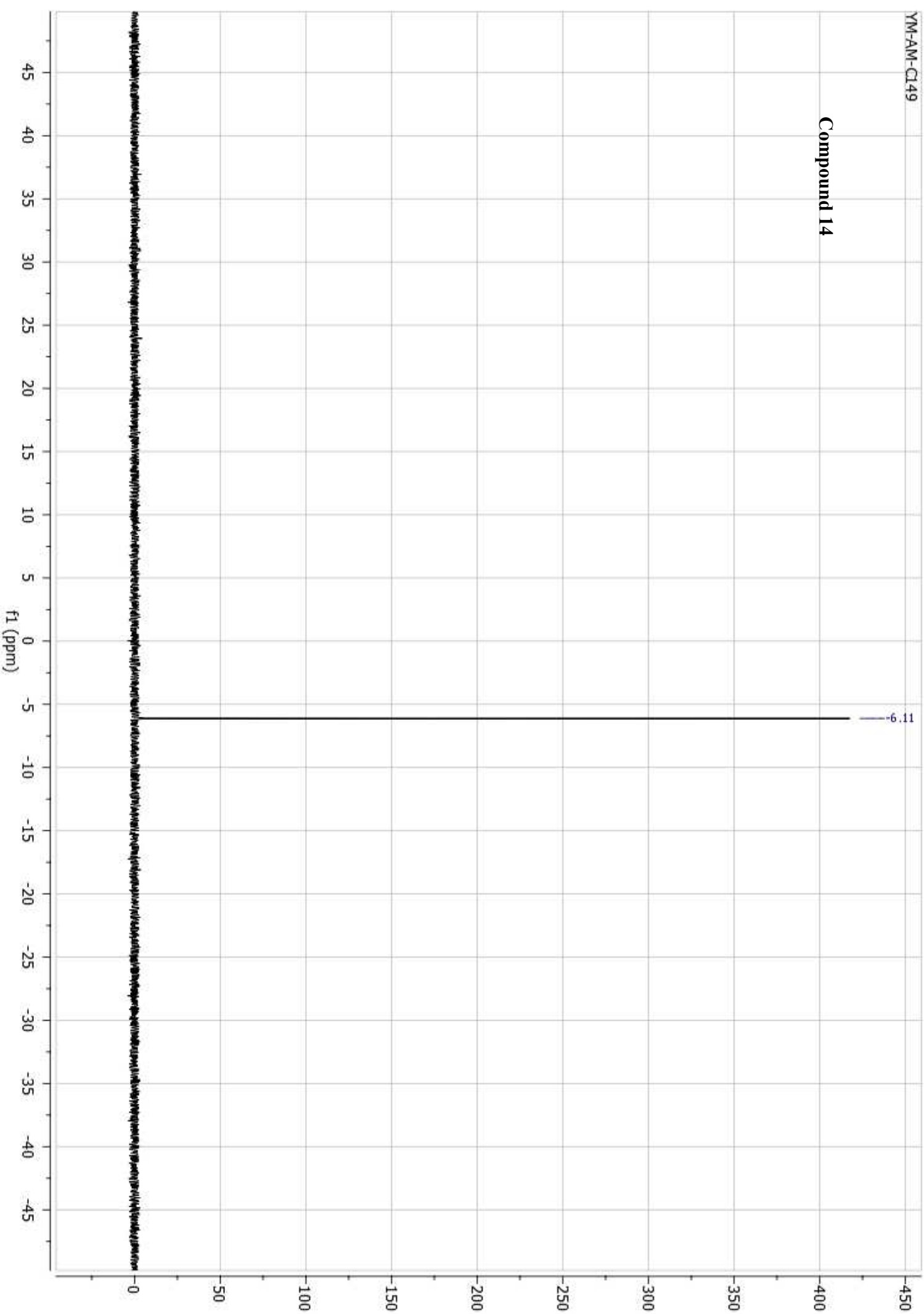
Compound 14



Compound 14



Compound 14





Analysis Report

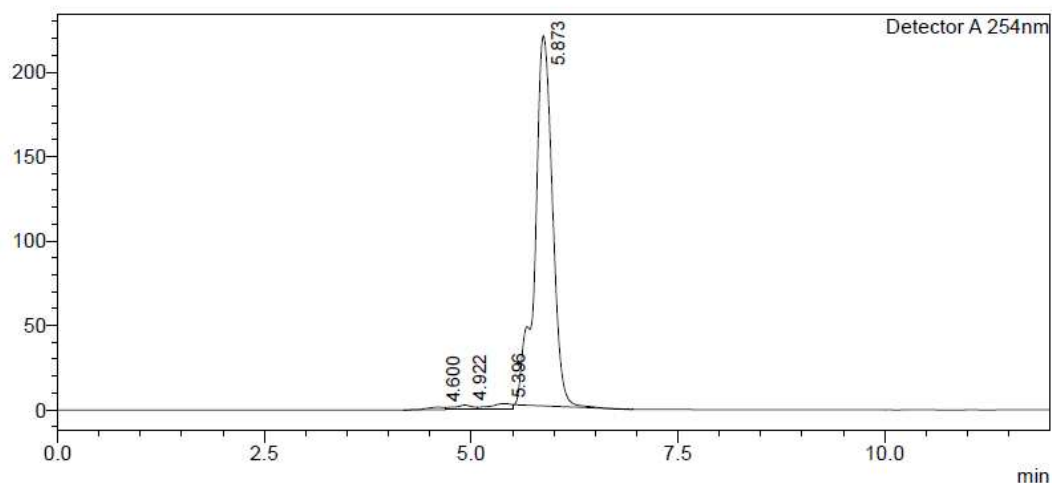
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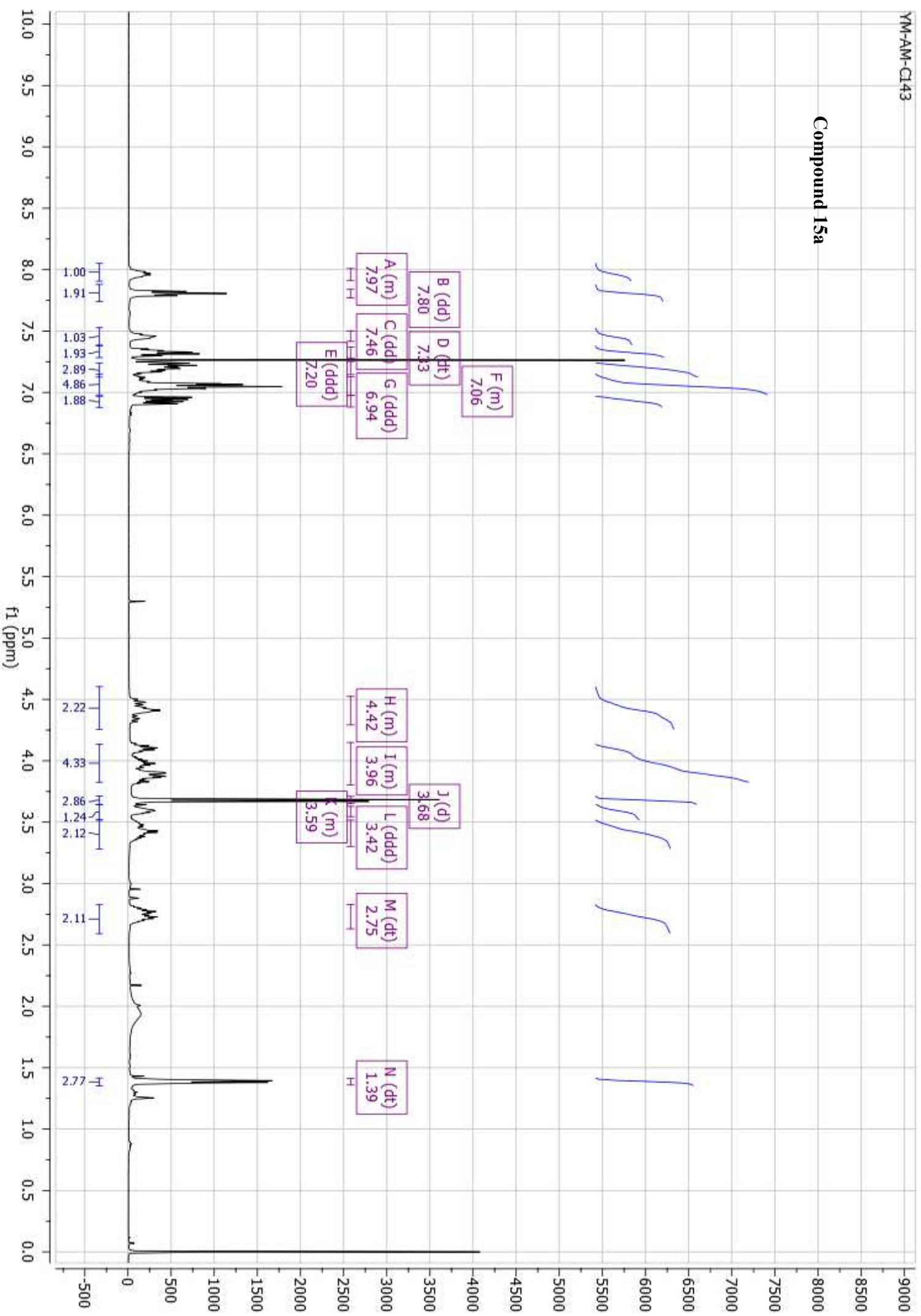
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Compound 14

Compound 15a



YM-AM-CL43

Compound 15a

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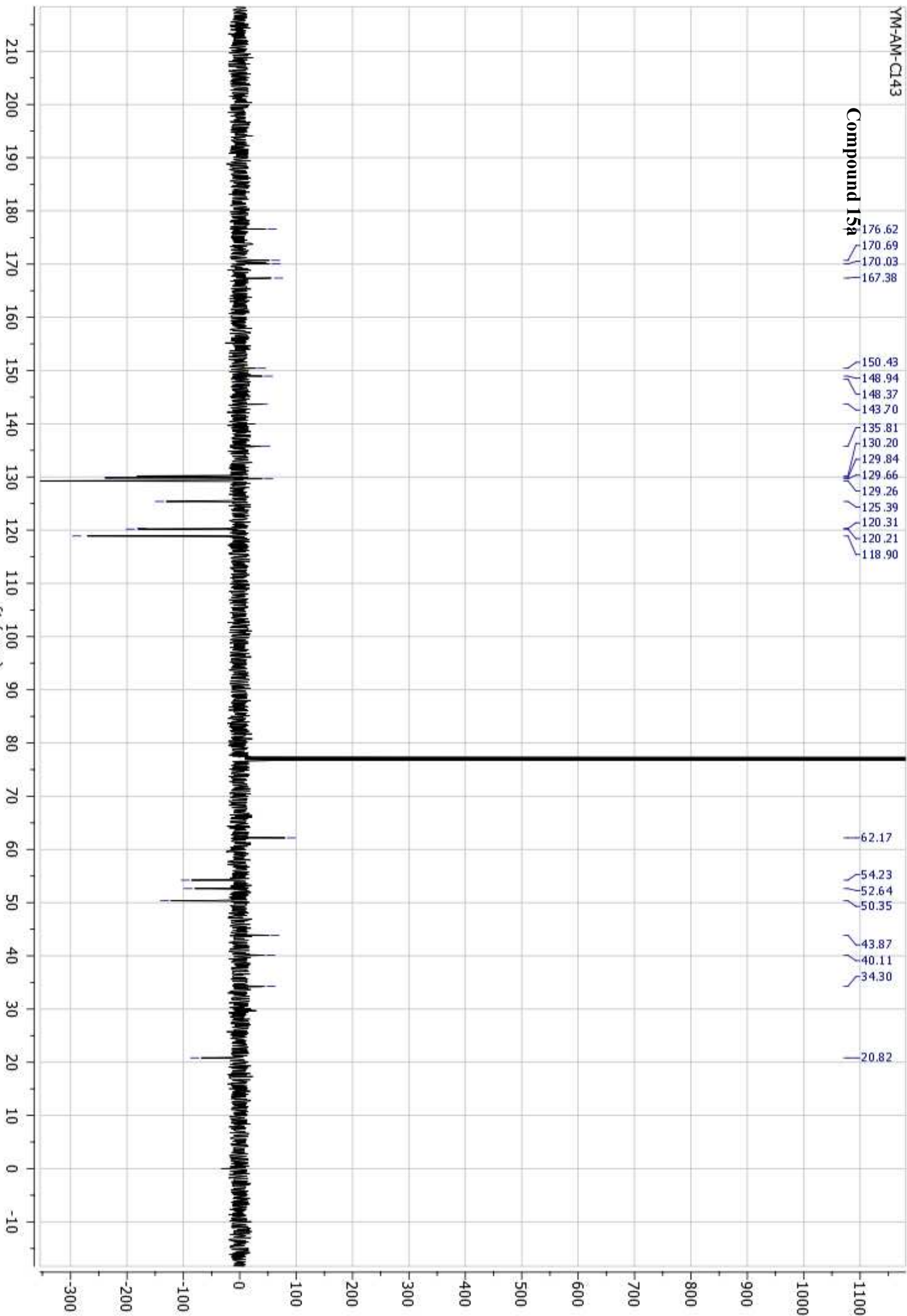
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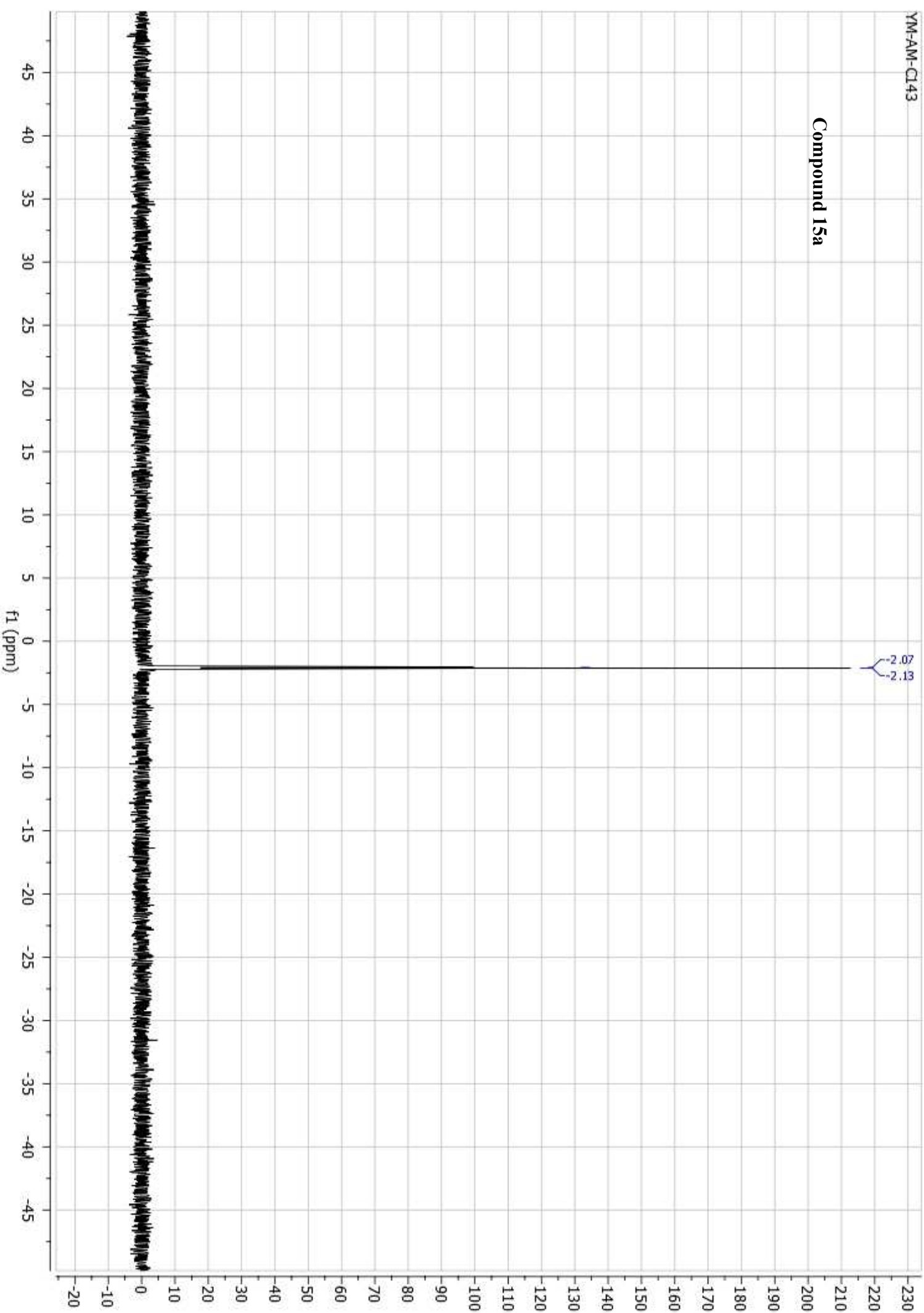
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Compound 15a





Analysis Report

<Sample Information>

Sample Name : AM(143)2
 Sample ID : AM(143)2
 Data Filename : 13112018_AM(143)2_001.lcd
 Method Filename : method 0.5mlmin 251018.lcm
 Batch Filename : AM(143)2.lcb
 Vial # : 1-20
 Injection Volume : 10 uL
 Date Acquired : 13/11/2018 13:45:57
 Date Processed : 30/11/2018 17:15:37

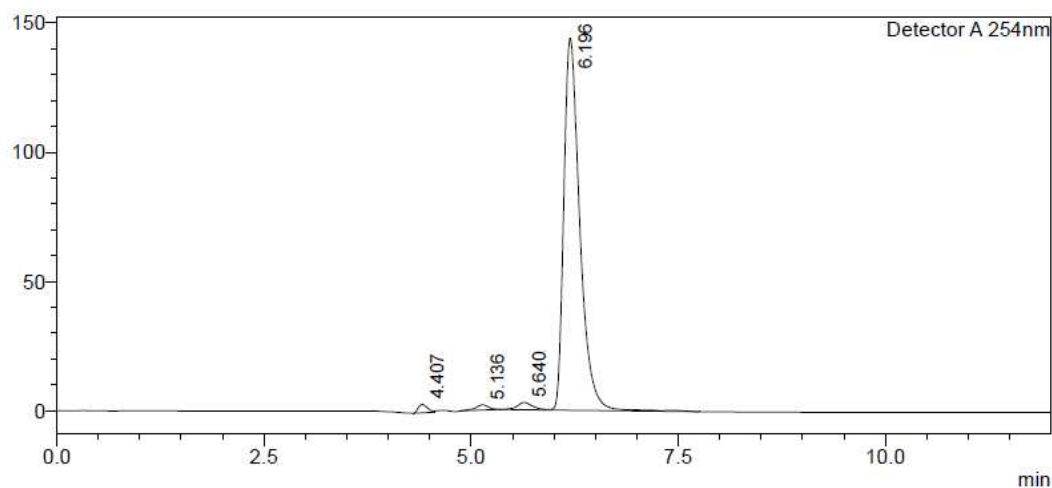
Sample Type : Unknown

Acquired by : System Administrator

Processed by : System Administrator

<Chromatogram>

mV



<Peak Table>

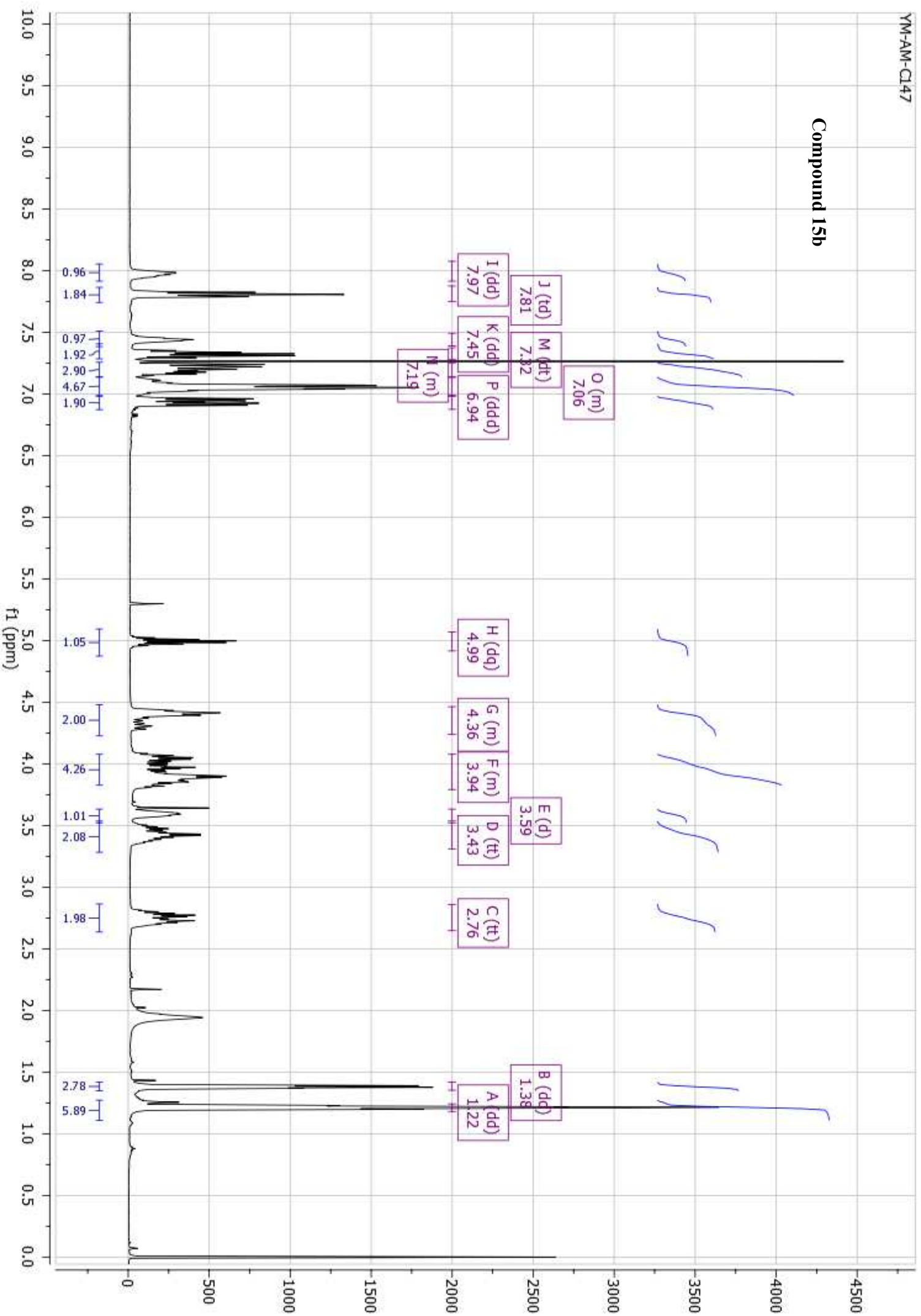
Detector A 254nm

Area	Area%	Time	Mark	Name
25563	1.267		M	
22210	1.101		M	
36156	1.793		M	
1933033	95.839		SV M	
2016962	100.000			

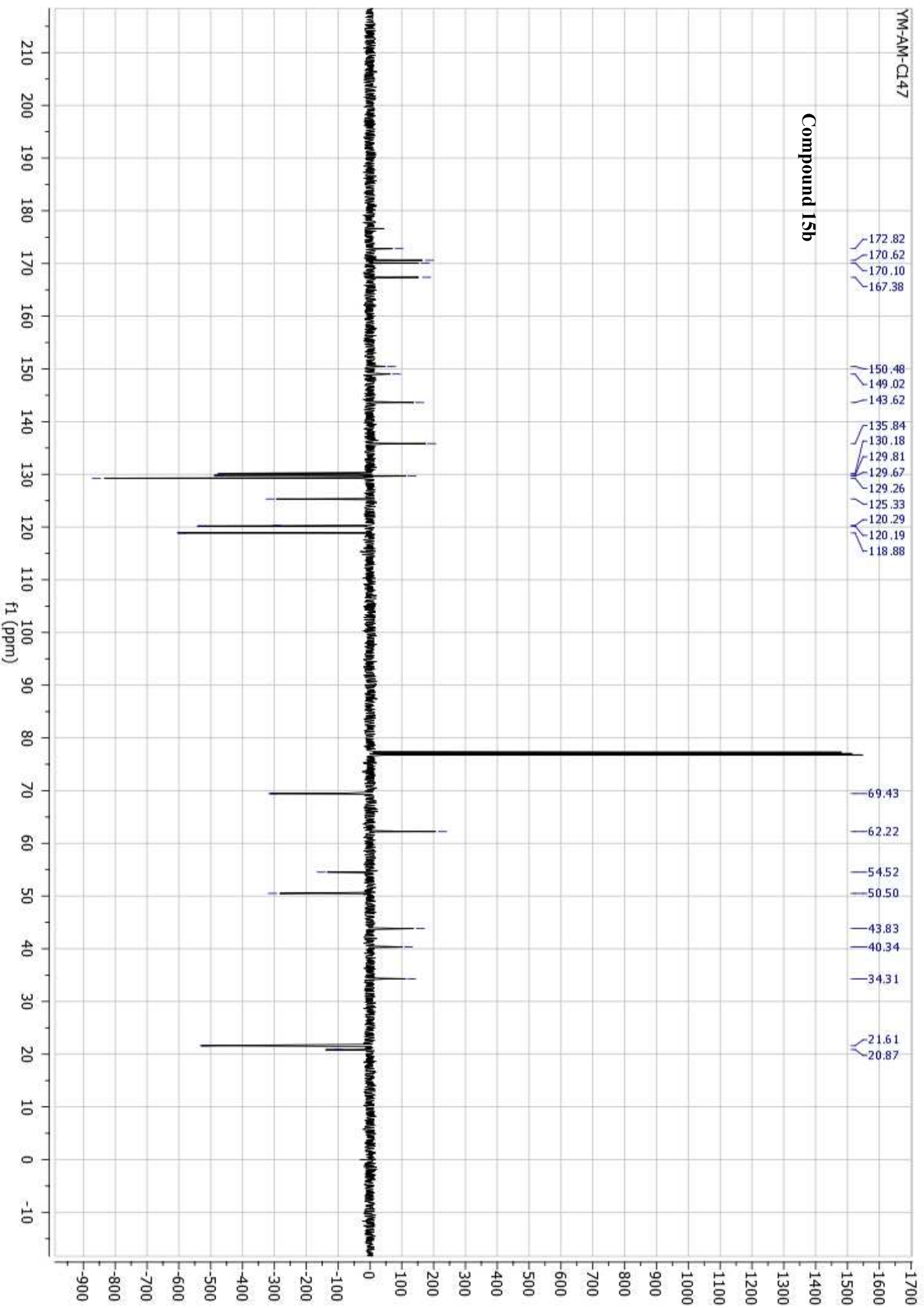
Compound 15a

C:\LabSolutions\Data\YM\AM\13112018_AM(143)2_001.lcd

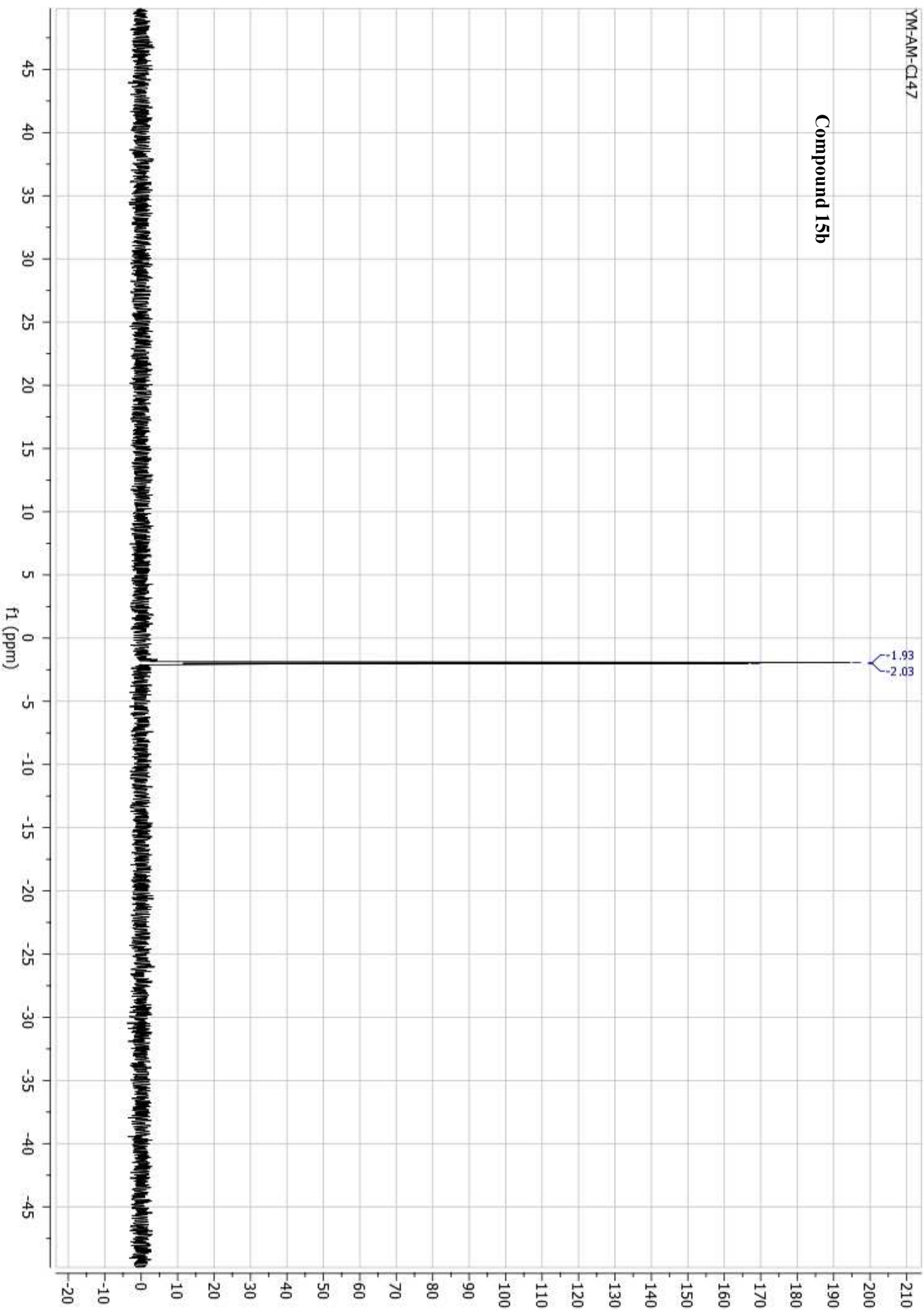
Compound 15b



Compound 15b



Compound 15b





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Analysis Report

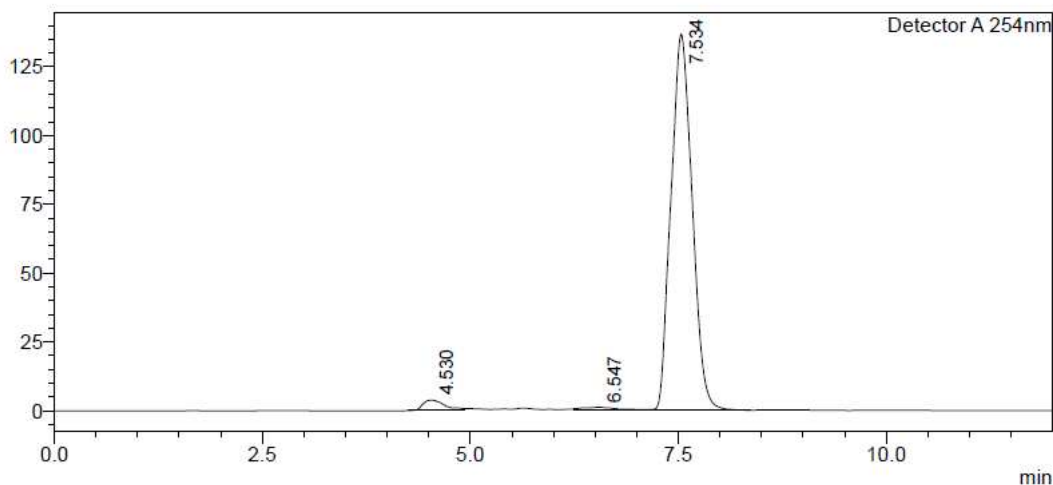
<Sample Information>

Sample Name : AM147
 Sample ID : AM147
 Data Filename : AM147.lcd
 Method Filename : method 0.5mlmin 251018.lcm
 Batch Filename : AM147.lcb
 Vial # : 1-1
 Injection Volume : 10 uL
 Date Acquired : 04/12/2018 14:45:30
 Date Processed : 04/12/2018 14:57:33

Sample Type : Unknown
 Acquired by : System Administrator
 Processed by : System Administrator

<Chromatogram>

mV



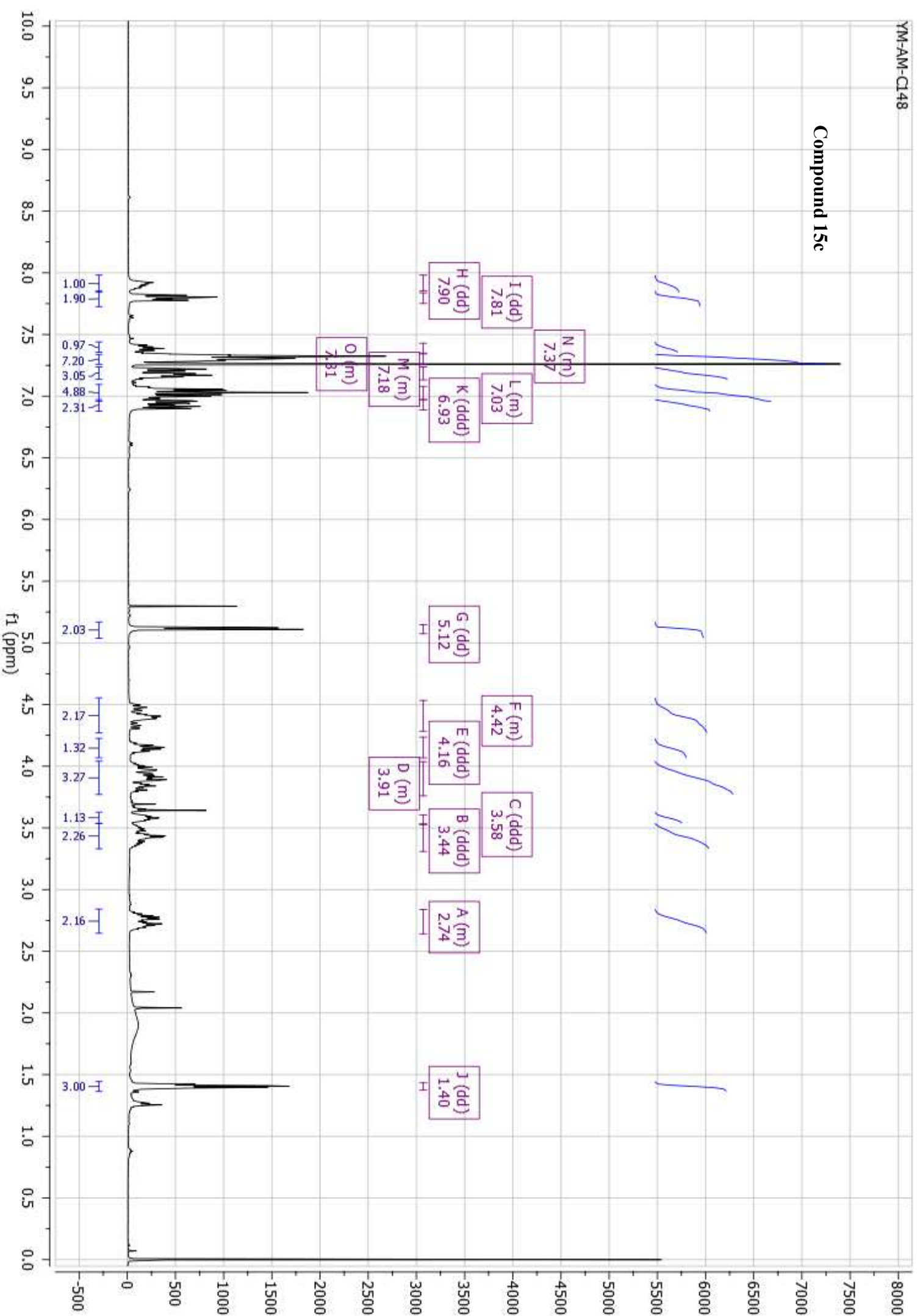
<Peak Table>

Detector A 254nm

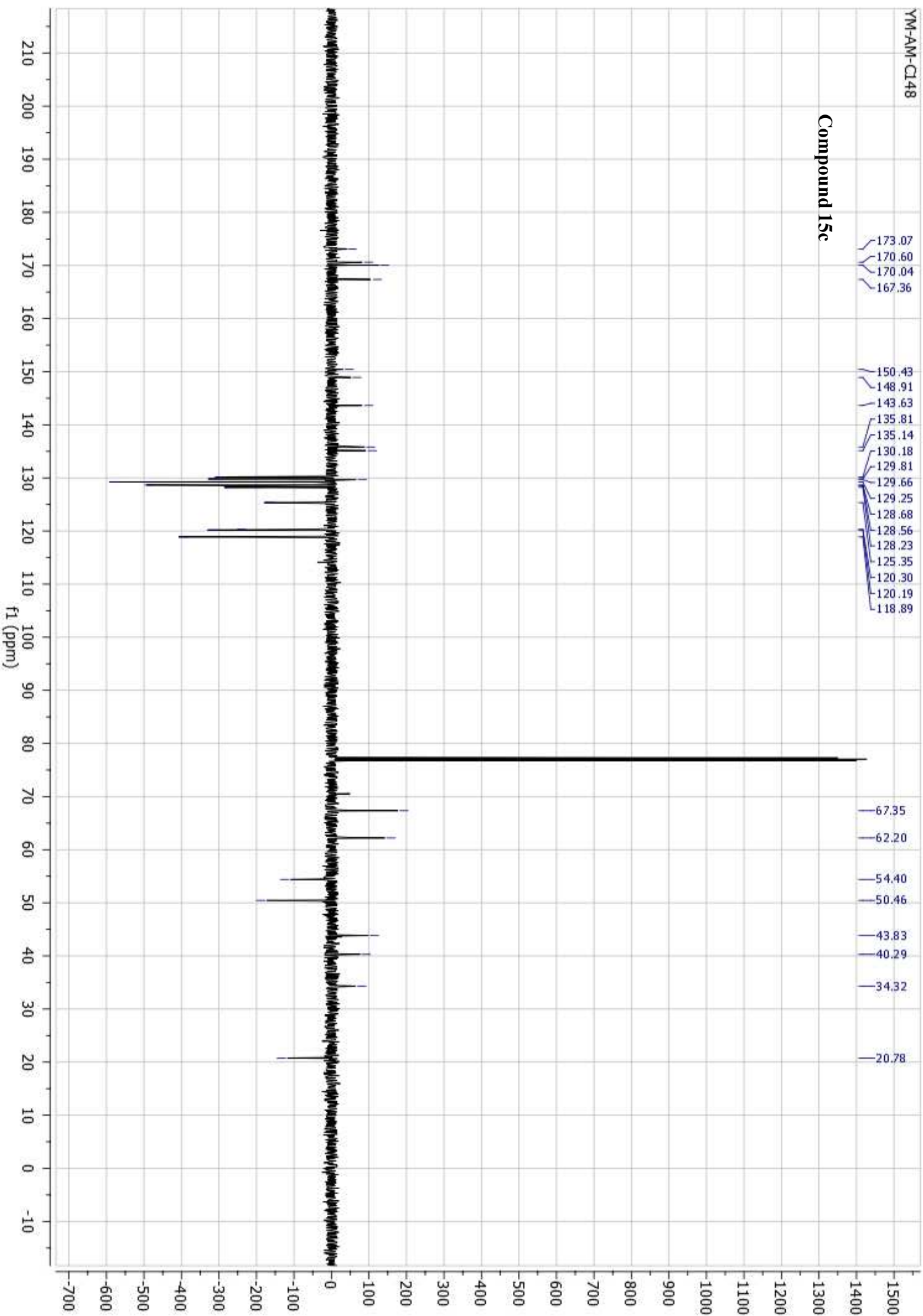
Peak#	Ret. Time	Area	Area%	Height	Unit	Mark	Name
1	4.530	71924	2.831	3763		M	
2	6.547	28904	1.138	1172		V M	
3	7.534	2439986	96.032	136755		V M	
Total		2540814	100.000	141691			

Compound 15b

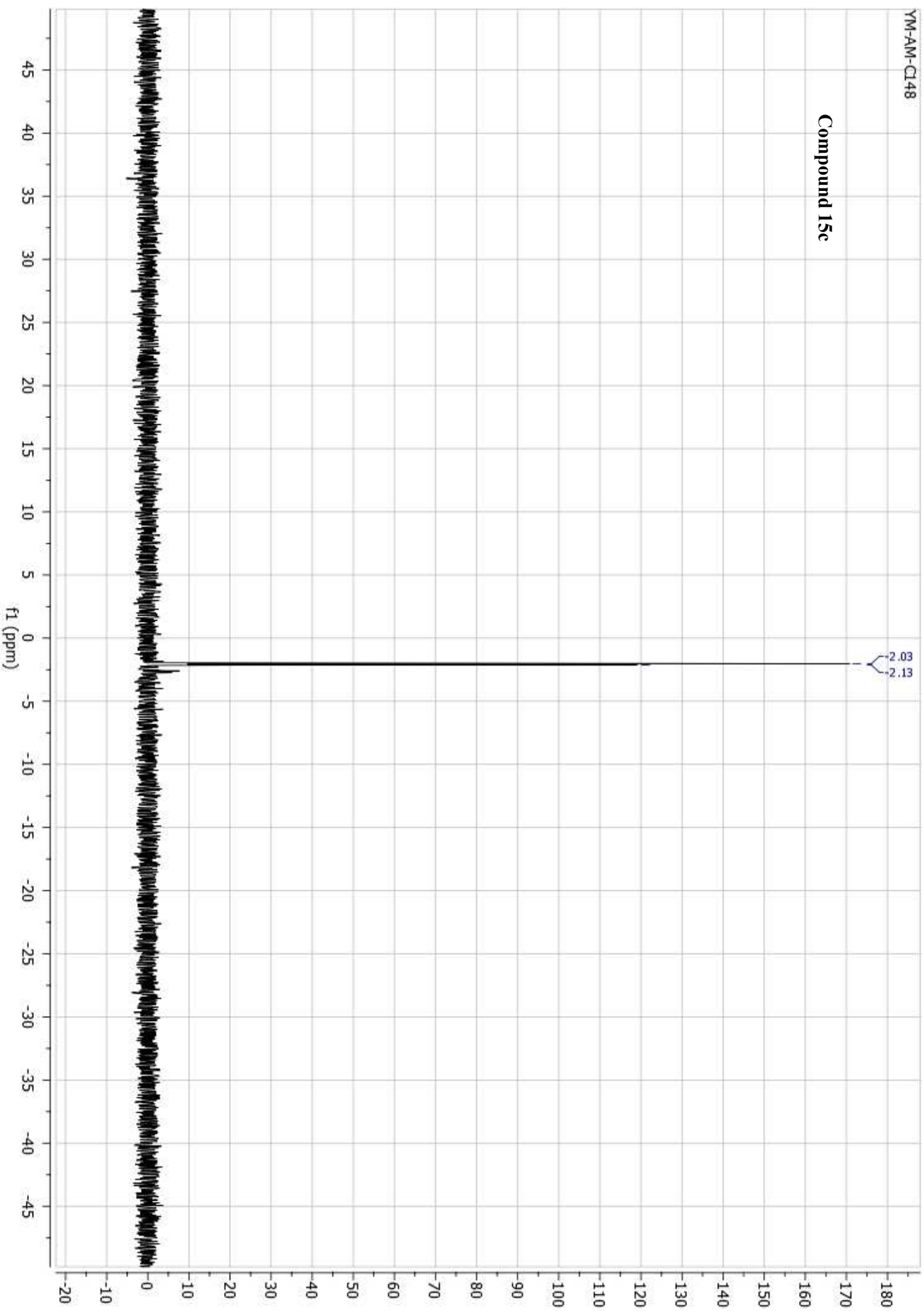
Compound 15c



Compound 15c



Compound 15c



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<Sample Information>

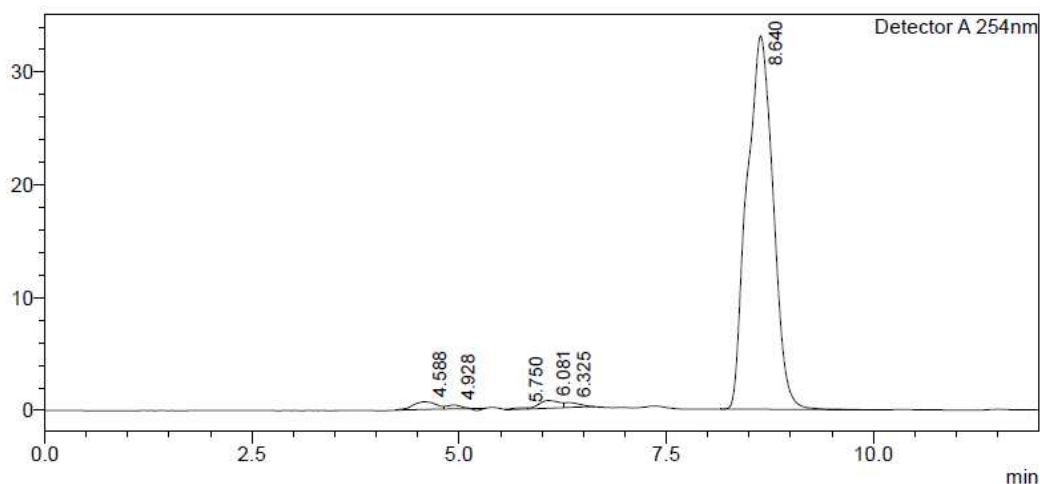
Sample Name : AM148
 Sample ID : AM148
 Data Filename : AM151.lcd
 Method Filename : method 0.5mlmin 251018.lcm
 Batch Filename : AM148-3.lcb
 Vial # : 1-1
 Injection Volume : 10 uL
 Date Acquired : 04/12/2018 14:32:42
 Date Processed : 04/12/2018 14:44:43

Sample Type : Unknown

Acquired by : System Administrator
 Processed by : System Administrator

<Chromatogram>

mV



<Peak Table>

Detector A 254nm

Peak#	Ret. Time	Area	Area%	Height	Unit	Mark	Name
1	4.588	13268	1.736	677		M	
2	4.928	3334	0.436	346		V M	
3	5.750	1748	0.229	124			
4	6.081	12005	1.571	684		V	
5	6.325	5724	0.749	445		V M	
6	8.640	728050	95.278	33093			
Total		764129	100.000	35369			

Compound 15c